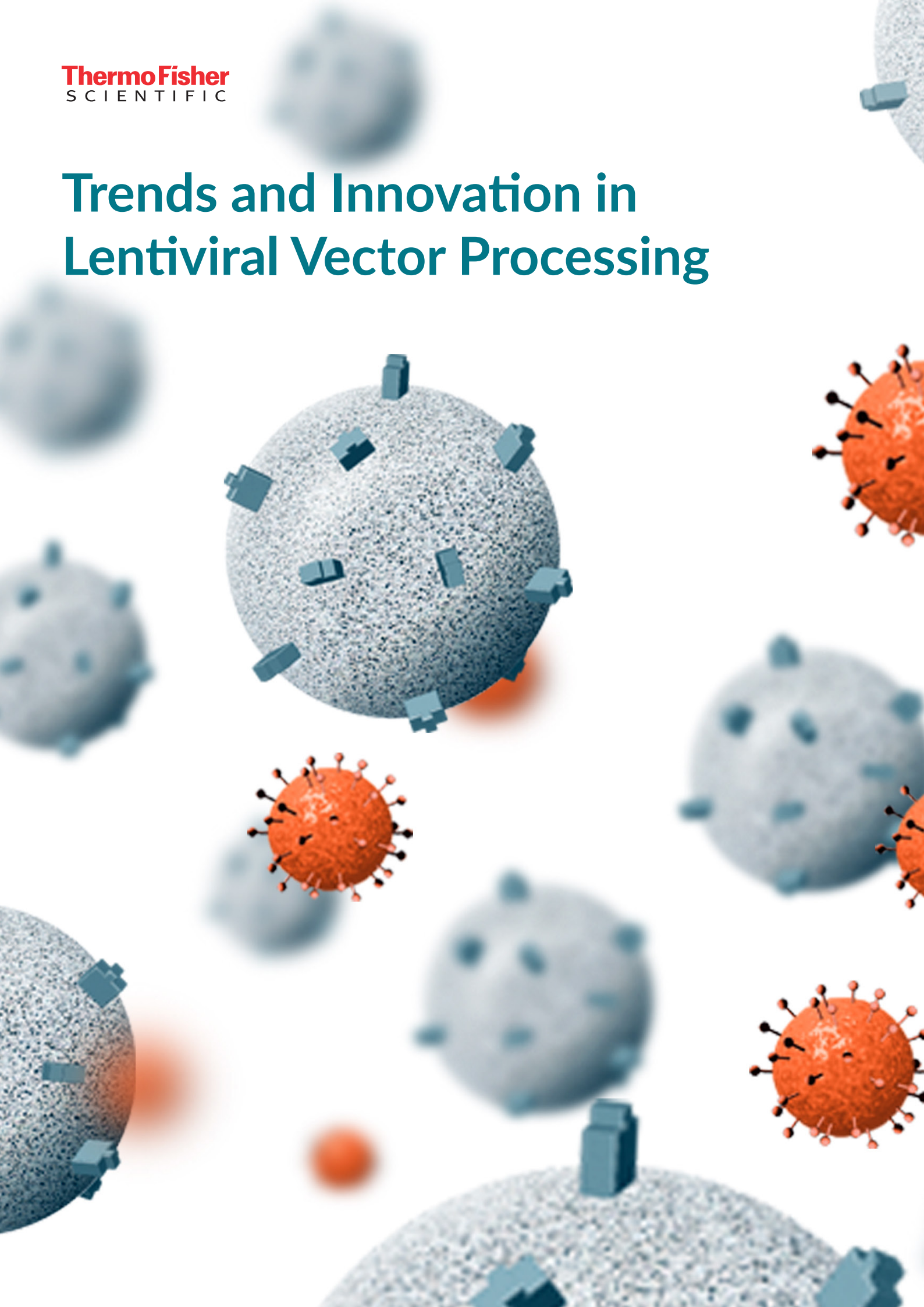
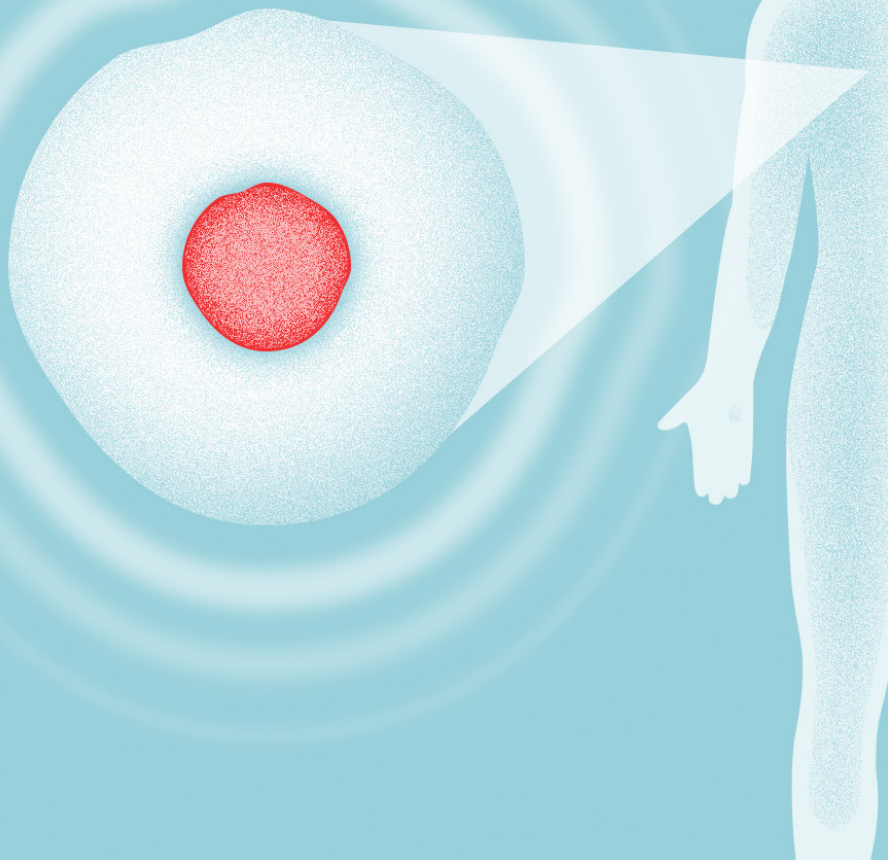
ThermoFisher SCIENTIFIC

# Trends and Innovation in Lentiviral Vector Processing



ThermoFisher SCIENTIFIC

# Advance lentiviral purification



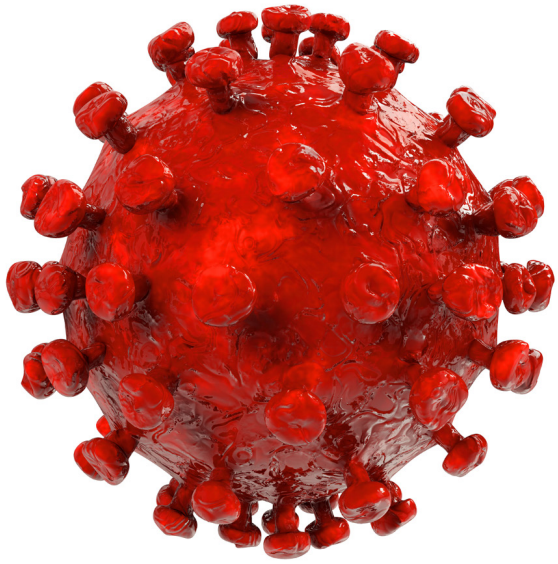
## Simplify your lentiviral purification process

The development pipeline of lentiviral particle-based therapies is growing and so is the need for efficient and productive production tools.

Introducing, Thermo Scientific™ CaptureSelect™ Lenti VSVG affinity matrix, specifically designed for the purification of VSV-G pseudotyped lentivirus particles from suspension cultivations. This resin offers an efficient and scalable purification method for lentiviral particles in combination with gentle elution conditions to retain viral infectivity.

### Why choose the CaptureSelect™ Lenti VSVG affinity matrix resin?

- It's designed to bind to VSV-G pseudotyped Lentiviral vector particles
- Achieve high recovery and purity in a single step
- It offers gentle elution conditions, based on Arginine, to retain infectivity of the lentivirus particles
- It is a scalable affinity purification method based on an agarose base-bead
- Non-animal derived



**Access the full article on Implementation of Novel Affinity Ligand for Lentiviral Vector Purification >>**

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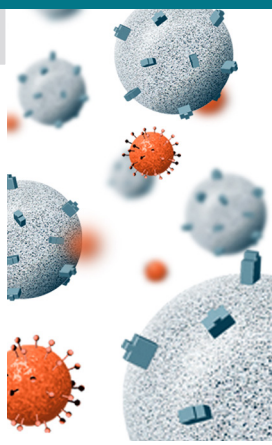
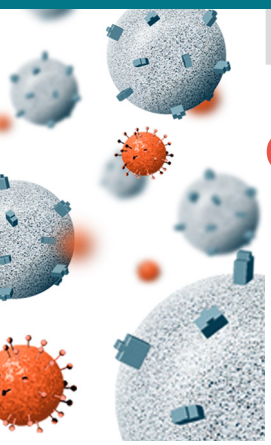
CELL & GENE THERAPY INSIGHTS



# Trends and Innovation in Lentiviral Vector Processing



CELL & GENE THERAPY INSIGHTS

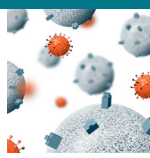
# Trends and innovation in lentiviral vector processing

**David McCall,** Editor, *Cell & Gene Therapy Insights*

# FOREWORD

Having become established more than a decade ago as the primary option for transducing CAR-T and other engineered cell therapies, lentiviral (LV) vectors continue to push back the barriers of what is possible in the cell and gene therapy world. Advances in engineering—in particular, to address safety and efficiency concerns—mean that today, LV vectors are increasingly being utilized in both the in vivo and the *ex vivo* gene therapy settings. Meanwhile, on the process and analytical technology front, the LV vector field has become a key area of focus for the development of much-needed bespoke tools. These innovations are aiding in the reduction of the high cost of goods traditionally associated with LV production, whilst also boosting the quality and consistency of manufacture.

BioInsights and Thermo Fisher Scientific are proud to present this unique curated collection of articles, videos, and posters dedicated to the LV processing field. Here you will find valuable insights into overriding trends in the evolution and application of LV vectors, as well as answers to your specific questions regarding the optimal approach to manufacturing and in particular, LV purification.



LIVE30 TRANSCRIPT

# Advancing the purification of VSV-G pseudotyped lentiviral vectors by using affinity chromatography

Pim Hermans & Frank Detmers

Cell and gene therapy vectors derived from lentivirus (LV) offer unique advantages over more conventional retroviral gene delivery systems. Considering the ability to integrate the host cell genome, LV vectors have become effective tools to transduce both dividing and non-dividing cells, thereby providing long-term stable gene expression. With a growing pipeline of LV particle-based therapies comes a prominent need for more efficient manufacturing processes that are meeting the demand of functional LVs required for clinical trials. Despite the manufacturing process improvements achieved over recent years, current unit operations are still unable to reverse the significant loss of biological LV particles during the downstream process. One of the major challenges has been the development of a truly selective affinity chromatography resin that can bind the viral envelope and simultaneously allow the preservation of its biological activity during elution. This article describes a new affinity resin, suitable for the purification of VSV-G pseudotyped lentivirus particles.

*Cell & Gene Therapy Insights* 2023; 9(4), 389–397

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## LENTIVIRUS PURIFICATION CHALLENGES

With the elevated use of lentivirus (LV) vector-based therapies in clinical trials, there is an increasing demand for good quality, highly pure vectors. Nevertheless, there is a plethora of purification challenges to overcome in order to reach the desired purity levels needed for clinical use.

LV is an enveloped virus that is produced using mammalian cell lines, such as HEK293 cells. One of the major challenges process developers face is separating LV vectors from

the large variety of closely-related product forms in the feedstock – for instance, exosomes. LV particles and extracellular vesicles such as exosomes follow a similar expression route in the cell. Consequently, the production of LV vectors yields a number of variations on both the vector and the exosomes, which is illustrated in Figure 1. These product-related impurities need to be removed from the final product.

A further challenge is the separation of particles with and without a genetic payload. Besides a very complex feedstock, LV vectors are relatively unstable. Therefore, sheer stress, high salt concentrations, and high osmolarity should all be avoided. In addition, only a narrow range in pH and temperature can be used when handling these particles.

The combination of these factors makes finding a suitable and efficient purification strategy challenging. Current processes report total recoveries of approximately 30% or less.

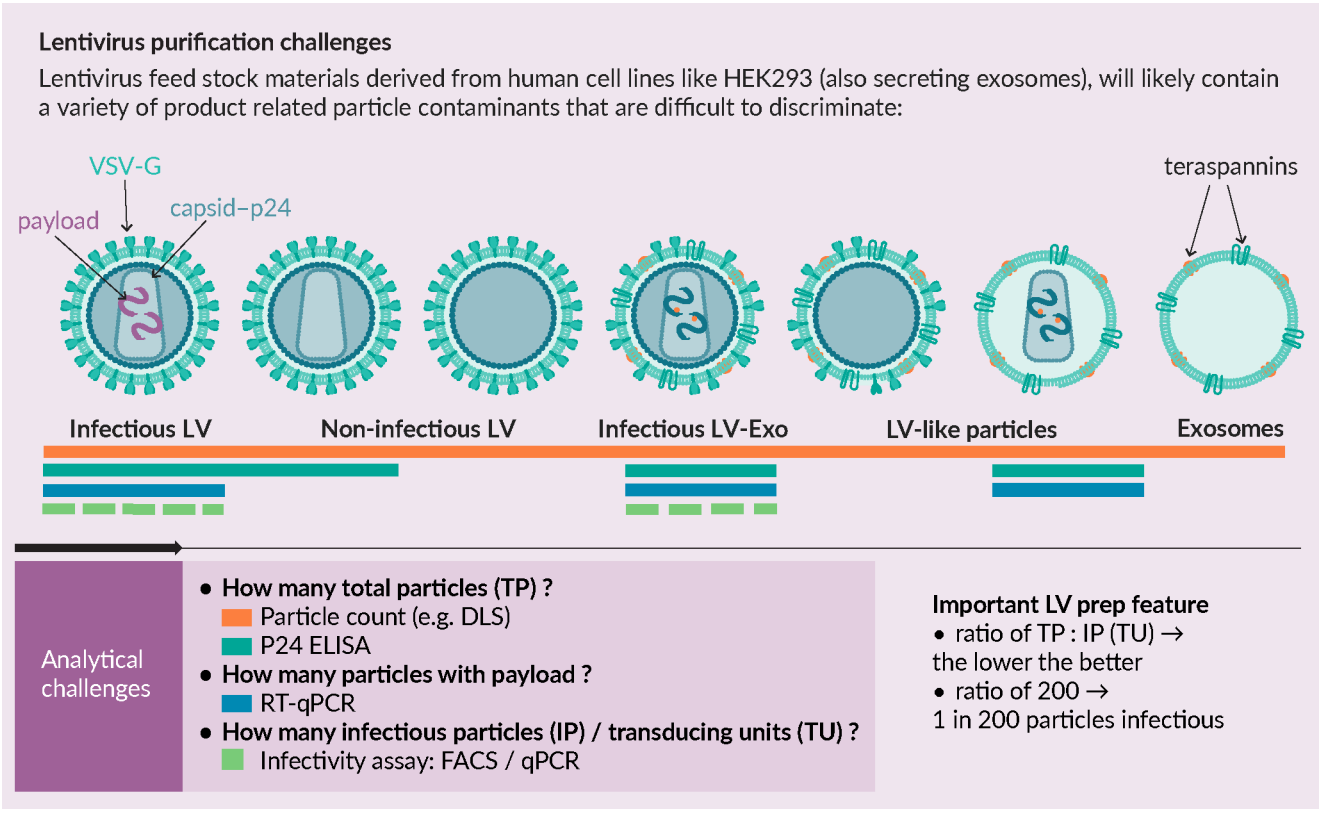
To determine both the quality of the feedstock and required steps of the purification process, it is essential to have the correct analytics in place. Important factors are the total number of particles (TP), the amount of particles with an effective payload (IP), and the ratio between these two groups (TP:IP). Figure 1 shows an overview of the various analytical assays and how they can discriminate between the different particles present in the cell culture feed or purification samples.

## AFFINITY RESIN DEVELOPMENT USING CAPTURESELECT™ TECHNOLOGY

To overcome the challenges in LV purification, an affinity resin targeting the VSV-G membrane protein was developed using

FIGURE 1

**Overview of lentivirus purification challenges and the analytics associated with process development.**



the CaptureSelect technology and resin development process. CaptureSelect ligands are based on single-domain antibody technology. The ligands are developed using an extensive screening technology where final process conditions are already implemented during screening. Ligands are tested for specificity, mild elution conditions, and stability to allow use in chromatography processes. The final ligand is recombinantly expressed in a yeast production process, which is free of animal components. CaptureSelect products are used in late clinical-stage and commercial processes. Resins are developed in a variety of drug development areas such as antibodies, biosimilars, plasma proteins, and viral vectors. The preferred resin features for the Lenti VSVG resin are shown in Box 1.

Firstly, a library was created to identify binders to the VSV-G target protein. Secondly, ligands capable of binding the target were screened using a Surface Plasmon Resonance (SPR) array-based system to monitor the selectivity and the ability to release under mild elution conditions. Three ligands demonstrated good binding in the SPR assay and selectivity was confirmed using a

## BOX 1

**Preferred design features for the design of the CaptureSelect VSVG affinity matrix.**

**High purity and yield in a single capture step**

- Good HCP and DNA clearance
- Reducing the number of purification steps
- Suitable for cell clarified harvest (no concentration)

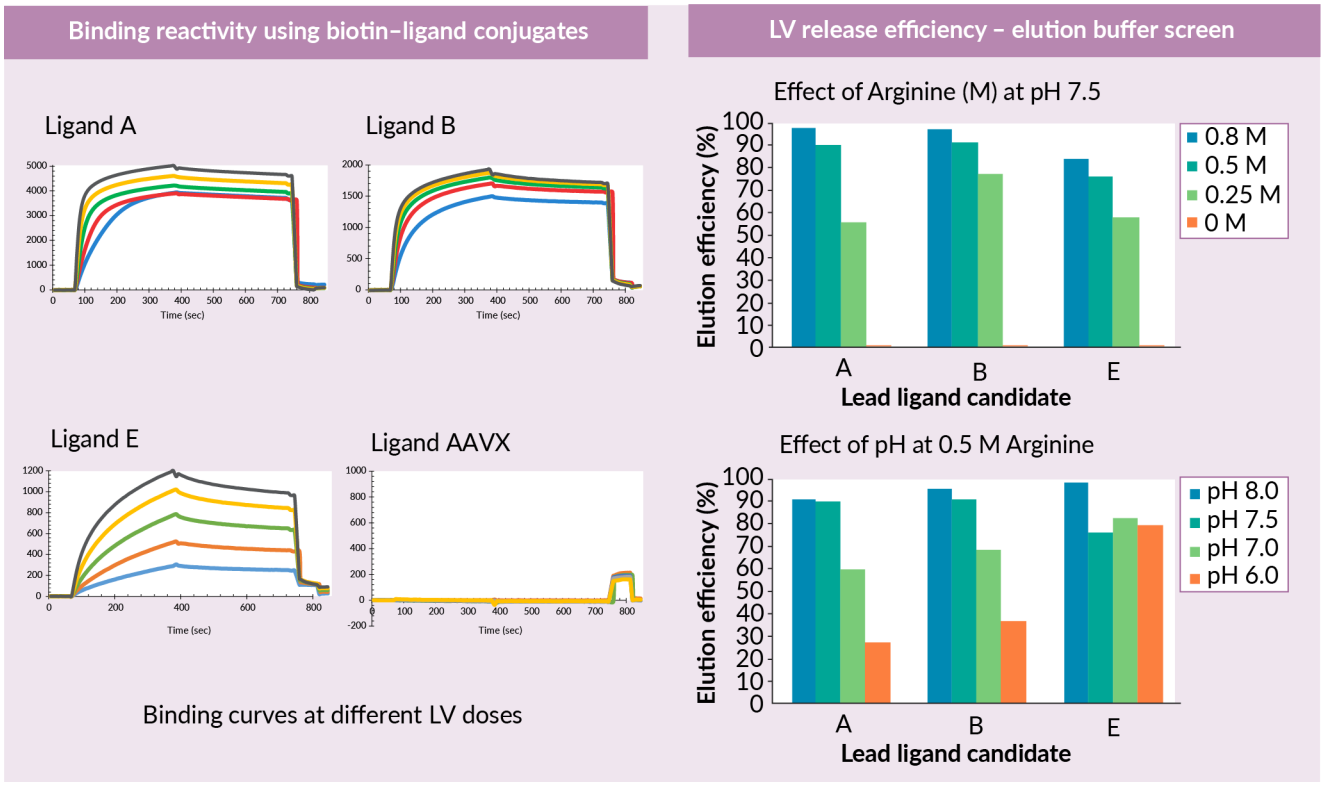
**Target release under mild elution conditions to retain LV infectivity**

- Good recoveries of active LV particles
- Improved TP:IP ratios

**Scalable**

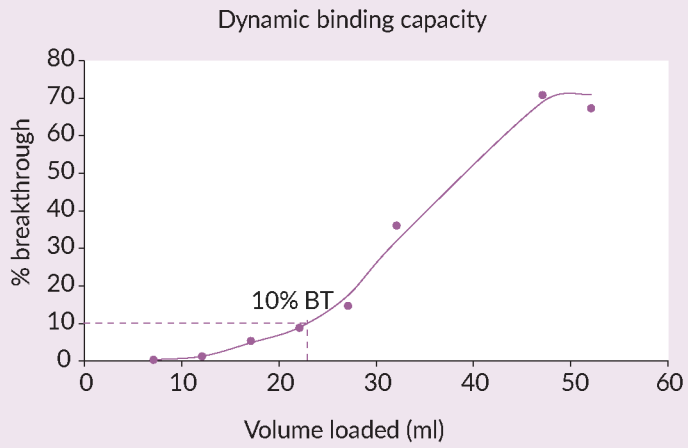
## FIGURE 2

**Ligand evaluation experiments showing SPR binding curves at different LV doses (left) and release efficiency using a mixed set of buffers and elution conditions (right).**



## FIGURE 3

**Graph showing DBC of the resin. 10% breakthrough is estimated at $1\times10^{11}$ particles/mL.**




**DBC is determined by P24 total particle ELISA**

| Fraction | Volume (mL) | TP/mL | $C/C_0$ |
|---|---|---|---|
| Start | | $3.98\times10^{9}$ | |
| 1 | 7.1 | $1.55\times10^{7}$ | 0.39 |
| 2 | 12.1 | $5.24\times10^{7}$ | 1.32 |
| 3 | 17.1 | $2.14\times10^{8}$ | 5.39 |
| 4 | 22.1 | $3.53\times10^{8}$ | 8.88 |
| 5 | 27.1 | $5.85\times10^{8}$ | 14.7 |
| 6 | 32.1 | $1.44\times10^{9}$ | 36.09 |
| 7 | 47.1 | $2.82\times10^{9}$ | 70.84 |
| 8 | 52.1 | $2.68\times10^{9}$ | 67.33 |

24.6 mL

- 10% breakthrough ($C/C_0$= 10%) estimated from the curve at 24.6 ml loading
- This relates to 9.78E10 total particles/ml resin (= $1\times10^{11}$)

non-related ligand binding to AAV (Figure 2, left). In addition, a concentration of 0.8 M Arginine at neutral pH was identified as a compatible elution buffer for VSV-G pseudotyped LV vectors (Figure 2, right).

After screening, three ligand candidates were expressed in a yeast production system and developed into resin prototypes, using different backbones. Resin prototypes were tested extensively in a small-scale chromatography set-up in order to determine a small selection of lead candidates for final resin development.

## LENTI-VSVG RESIN CHARACTERISTICS

Dynamic binding capacity (DBC) of the resin was determined using the p24 total particle ELISA. Results are shown in Figure 3. A feed containing $4\times10^{9}$ total particles/mL was loaded onto a 1 mL column and flow-through fractions were analyzed. Based on the results, a binding curve was plotted and the 10% breakthrough point was determined. These results show that the DBC of the resin is $1\times10^{11}$ total particles/mL resin.

Next, purification conditions were determined in two consecutive runs on a 10 mL chromatography column, using 200 mL load material, a flowrate of 150 cm/h, and 2 min contact time. The feed was endonuclease treated, followed by a clarification on a 0.4 µm filter and direct loading on the column. Column equilibration was performed using a 50 mM HEPES buffer at pH 7.5, containing 150 mM NaCl. Elution was performed using the same HEPES buffer containing 0.8 M Arginine. After the run, a strip of the column was performed using 50 mM sodium phosphate pH12. The chromatographic profile and a close-up of the elution peak are presented in Figure 4.

Fractions of the two chromatography runs were further analyzed to determine the ratio of total particles versus infectious particles.

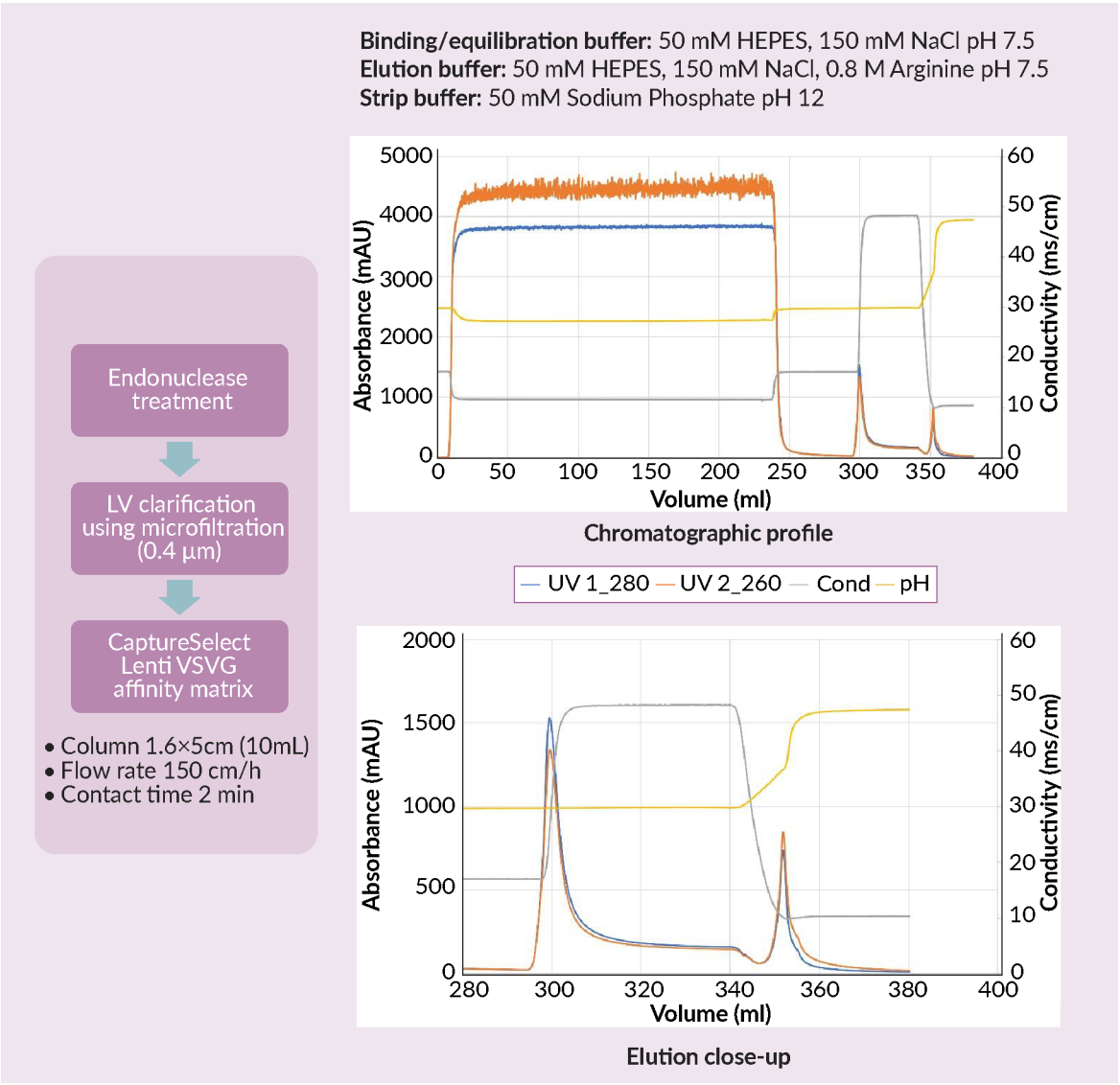
## TABLE 1

**Overview of total particles and infectious particles, and their ratio (TP:IP).**

| Sample | TP/mL | IP/mL | TP/IP ratio |
|---|---|---|---|
| 1. Feed | $1.10\times10^{10}$ | $7.98\times10^{7}$ | 138 |
| 1. Flow through | $3.25\times10^{8}$ | $8.30\times10^{5}$ | 392 |
| 1. Elution | $4.44\times10^{10}$ | $4.42\times10^{8}$ | 100 |
| 2. Feed | $1.11\times10^{10}$ | $9.00\times10^{7}$ | 123 |
| 2. Flow through | $1.28\times10^{9}$ | $5.45\times10^{6}$ | 235 |
| 2. Elution | $2.6\times10^{10}$ | $4.66\times10^{8}$ | 56 |

## FIGURE 4

**The chromatography process using the Lenti VSVG affinity matrix (left) and the chromatographic profile (right).**




The results, demonstrated in Table 1, reveal a five-fold enrichment of the infectious particles in the final elution fraction and a decreasing TP:IP ratio. In addition, host cell protein (HCP) and DNA removal, along with total recovery of the elution fractions, was determined. Total recovery of the LV particles was between 50–60% and HCP and DNA impurity removal was considered to be highly efficient; between 80–99% (Table 2).

## CONCLUSION

The CaptureSelect Lenti VSVG affinity matrix is designed to help increase productivity and efficiency in the downstream process of VSV-G pseudotyped lentiviral vectors from suspension culture. It provides gentle elution conditions, based on Arginine, to retain infectivity of the LV particles. Furthermore, the resin is a scalable affinity purification method without animal-derived components.

TABLE 2

**Final recovery, HCP, and total DNA removal from the elution fractions of two chromatography runs.**

| Sample | Volume (mL) | IP/mL | TU (Transduction units) | Recovery | HCP removal | Total DNA removal |
|---|---|---|---|---|---|---|
| 1. Feed | 250 | $7.98\times10^{7}$ | $1.99\times10^{10}$ | | | |
| 1. Flow through | 258 | $8.30\times10^{5}$ | $2.14\times10^{8}$ | | | |
| 1. Elution | 22.5 | $4.42\times10^{8}$ | $9.95\times10^{9}$ | 49.9% | 98.7% | 80.2% |
| 2. Feed | 230 | $9.00\times10^{7}$ | $2.07\times10^{10}$ | | | |
| 2. Flow through | 240 | $5.45\times10^{6}$ | $1.31\times10^{9}$ | | | |
| 2. Elution | 25.6 | $4.66\times10^{8}$ | $1.19\times10^{10}$ | 57.7% | 97.1% | 96.5% |

# ASK THE EXPERTS

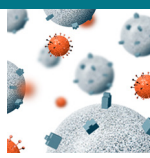
**Abgail Pinchbeck,** Assistant Editor, BioInsights speaks to (pictured left to right) **Pim Hermans,** Director of Ligand Discovery for BioProduction Group, Thermo Fisher Scientific and **Frank Detmers,** Director of Ligand Application for CaptureSelect, Thermo Fisher Scientific

**Q** Can you expect performance differences between suspension and adherent cultured feedstocks?

**PH:** Performance differences can be expected. It depends on the quality of the material. In suspension cell feeds, the ratio of total particles versus infectious particles is quite low. In adherent cell feed stocks, where the ratio can be approximately 1000:1, the composition of the material is quite different and the number of actual infectious particles is relatively low compared to the suspension cell feeds. Purification will therefore be more challenging, even for affinity solutions.

**Q** Can the purity level of the elution fraction regarding host cell proteins and residual DNA be further optimized?

**FD:** We have seen in ongoing customer evaluations that increasing the NaCl concentration between 300–450 mM for an intermediate wash buffer before eluting can help in further reducing these types of impurities. When you implement an affinity resin, the wash conditions and elution conditions are the steps that often need some optimization.



## Q Does the resin also work for non-VSV-G pseudotyped lentiviruses?

**PH:** For the development of the resin, we focused on a specific protein that is expressed by lentivirus. In this case, the VSV-G protein was chosen, which means the resin only binds to VSV-G pseudotyped lentivirus particles.

## Q Are there any plans to make this research-use-only resin suitable for bioprocessing?

**FD:** There are plans for upscaling of the resin, making it suitable for bioprocessing. It is scheduled to be available by the end of this year. It will come together with all the support packages needed such as a ligand-leakage ELISA and a regulatory support file. In addition, we are planning to generate supplementary data.

## BIOGRAPHIES

**PIM HERMANS** leads the ligand discovery team at Thermo Fisher Scientific. Antibodies and affinity research have been a common theme throughout his whole career. After receiving his Bachelors degree in Biochemistry he proceeded to work at Holland Biotechnology where he carried out research on the production and purification of recombinant cytokines and monoclonal antibodies. By joining Unilever-Bestfoods, Pim was one of the first scientists involved in the early development and exploration of camelid derived single domain antibodies (VHHs). He joined the Bio Affinity Company (BAC, now part of Thermo Fisher Scientific) in 2003. As head of the Ligand Discovery Department Pim is responsible for the development of VHH based affinity ligands for applications in process – and analytical affinity chromatography. Through the introduction of new selection and screening methodologies he and his team enabled rapid development of affinity purification - and detection tools serving a broad variety of targets.

**FRANK DETMERS** received his PhD at the department of Molecular Microbiology of the University of Groningen (The Netherlands). From 2001 until 2004 he worked as a post-doctoral researcher at the Department of Cell Physiology at the Nijmegen Center of Molecular Life Sciences (NCMLS, Nijmegen, The Netherlands). He joined BAC BV (Leiden, The Netherlands) in 2004 and the focus of his work is immobilization of affinity ligands on solid supports and the development of new applications of the CaptureSelect ligands. Currently, Frank is director of ligand application for CaptureSelect at ThermoFisher. His work is focusing on the development of new purification tools in the field of antibodies, therapeutic proteins, and gene and cell therapy.

## AFFILIATIONS

**Pim Hermans**
Director of Ligand Discovery for BioProduction Group,
Thermo Fisher Scientific

**Frank Detmers, PhD**
Director of Ligand Application for CaptureSelect,
Thermo Fisher Scientific

## AUTHORSHIP & CONFLICT OF INTEREST

**Contributions:** All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

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**Publication date:** May 10 2023.

# Using affinity chromatography for the purification of Lentiviral particles

Pim Hermans, Frank Detmers, Sandra Bezemer & Laurens Sierkstra
Thermo Fisher Scientific, Leiden, the Netherlands

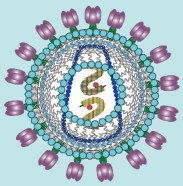
## Introduction

Cell therapy vectors derived from lentiviruses offer many potentially unique advantages over more conventional retroviral gene delivery systems. Most important is their ability to provide long-term and stable gene expression and to infect non-dividing cells, such as neurons. The development pipeline of lentiviral particle-based therapies is growing and so is the need for efficient and productive production tools

Here we present a new affinity chromatography resin, the CaptureSelect™ Lenti VSVG affinity matrix, specifically designed for the purification of VSV-G pseudotyped lentivirus particles from suspension cultivations. The resin offers an efficient and scalable purification method for lentiviral particles in combination with gentle elution conditions to retain viral infectivity.

## CaptureSelect Lenti VSVG affinity matrix

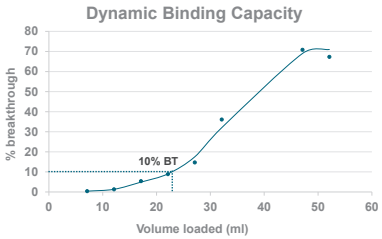
- **Based on CaptureSelect™ single-domain antibody technology**
- **Designed to bind VSV-G pseudotyped Lentiviral vector particles**
- **High recovery and purity in a single step**
- **Gentle elution conditions, based on Arginine, to retain infectivity of the lentivirus particles**
- **A scalable affinity purification method based on an agarose base-bead**
- **Non-animal derived**



**Designed to help increase productivity and efficiency in the downstream process of lentiviral vectors**

## Resin performance

### Dynamic Binding Capacity (DBC) study – 1mL column




| Fraction | Volume (mL) | TP/mL | C/C0 |
|---|---|---|---|
| Start | | 3.98E9 | |
| 1 | 7.1 | 1.55E7 | 0.39 |
| 2 | 12.1 | 5.24E7 | 1.32 |
| 3 | 17.1 | 2.14E8 | 5.39 |
| 4 | 22.1 | 3.53E8 | 8.88 |
| 5 | 27.1 | 5.85E8 | 14.7 |
| 6 | 32.1 | 1.44E9 | 36.09 |
| 7 | 47.1 | 2.82E9 | 70.84 |
| 8 | 52.1 | 2.68E9 | 67.33 |

24.6 mL

**Figure 1. DBC of the CaptureSelect Lenti VSVG affinity resin, determined by P24 total particle ELISA.** Lentivirus produced in HEK293 cells in suspension was loaded on a 1 ml (0.66x3 cm) column, equilibrated in 50 mM HEPES, 150 mM NaCl pH 7.5. The load material had a titer of $3.89E^9$ total particles/ml. The flow-through fractions were analyzed in a p24 ELISA to determine the breakthrough of the Lentivirus particles. 10% breakthrough of the Lentivirus particles was reached after loading 24.6 ml of the feed material, resulting in a DBC of the resin of $1E^{11}$ total particles/ml of resin. $C_0$ is the titer of the feed stock ($3.89E^9$ particles/ml), and C is the titer measured in the flow through fractions, the 10% breakthrough point was interpolated from the breakthrough curve.

✓ **DBC at 10% breakthrough is 1E11 particles/mL resin**

- 10% breakthrough ($C/C_0$= 10%) estimated from the curve at 24.6 ml loading
- This relates to 9.78E10 total particles/ml resin (= 1E11)

### Chromatography conditions – elution profile

**Process**

Endonuclease treatment

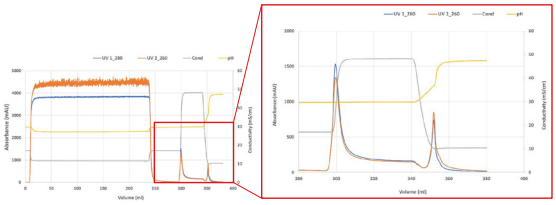
↓

LV clarification using microfiltration (0.4 µm)

↓

CaptureSelect Lenti VSVG affinity matrix

Column 1.6x 5cm (10mL)
Flow rate 150 cm/h
Contact time 2 min



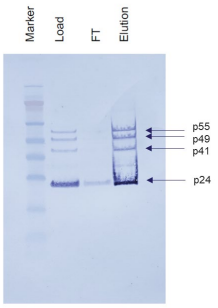
Chromatographic profile — Elution profile

**Figure 2. Chromatography conditions using a 10mL column and 250 mL clarified suspension harvest with a titer of 1E10 total particles/mL**
**Binding/equilibration buffer:** 50 mM HEPES, 150 mM NaCl pH 7.5
**Elution buffer:** 50 mM HEPES, 150 mM NaCl, 0.8 M Arginine pH 7.5
**Strip buffer:** 50 mM Sodium Phosphate pH 12

✓ **The resin demonstrates an efficient elution profile**

### Comparison of total particle to infectious particle ratios (n=2)

**P24 – WB pattern**




**Total particle (TP) and infectious particle (IP) ratio**

| Sample | TP/mL | IP/mL | TP/IP ratio |
|---|---|---|---|
| 1. Feed | 1.10E10 | 7.98E7 | 138 |
| 1. Flow through | 3.25E8 | 8.30E5 | 392 |
| 1. Elution | 4.44E10 | **4.42E8** | **100** |
| 2. Feed | 1.11E10 | 9.00E7 | 165 |
| 2. Flow through | 1.28E9 | 5.45E6 | 245 |
| 2. Elution | 2.6E10 | **4.66E8** | **71** |

➢ The eluted fractions show a more than 5-fold increase of the infectious particle concentration compared to the load

**Table 1. Total particles and infectious particle ratio.** Total particles are determined by p24 ELISA, infectious particles are determined through a cell infectivity assay. The data demonstrates an enrichment of infectious particles after affinity purification.

✓ **The concentration of infectious particles in the elution fraction has been enriched through purification using the Lenti VSVG resin**

### Recovery of infectious particles (n=2)

| Sample | Volume (mL) | IP/mL | TU (Transduction units) | Recovery | HCP removal | Total DNA removal |
|---|---|---|---|---|---|---|
| 1. Feed | 250 | 7.98E7 | 1.99E10 | | | |
| 1. Flow through | 258 | 8.30E5 | 2.14E8 | | | |
| 1. Elution | 22.5 | 4.42E8 | 9.95E9 | **49.9%** | **98.7%** | **80.2%** |
| 2. Feed | 230 | 9.00E7 | 2.07E10 | | | |
| 2. Flow through | 240 | 5.45E6 | 1.31E9 | | | |
| 2. Elution | 25.6 | 4.66E8 | 1.19E10 | **57.7%** | **97.1%** | **96.5%** |

**Table 2. Recovery of infectious particles determined through % of transduction units in the feed versus the elution fraction.** HCP removal was measured using ELISA, total DNA was measured using Picogreen™ fluorescent probe.

✓ **Recovery of infectious particles after purification using the Lenti VSVG resin is ~50-60%**

## Resin Characteristics

**MAIN RESIN CHARACTERISTICS**

**Matrix:** agarose-based, epoxide activated
**Average particle size:** 65 ± 10 µm
**Ligand:** CaptureSelect Lenti VSVG affinity ligand
**Ligand coupling method:** epoxide
**Binding capacity:** ~1E11 total particles/ml matrix
**Elution conditions:** 50 mM HEPES, 150 mM NaCl, 0.8 M Arginine pH 7.5
**Strip conditions:** 50 mM Sodium Phosphate pH 12
**Flow characteristics:** 50–200 cm/h (up to 2 bar)
**Formulation buffer:** 20% (v/v) ethanol

| Cat. Nr. | Product |
|---|---|
| 2943932005 | CaptureSelect™ Lenti VSVG Affinity Matrix 5mL |
| 2943932010 | CaptureSelect™ Lenti VSVG Affinity Matrix 10mL |
| 2943932050 | CaptureSelect™ Lenti VSVG Affinity Matrix 50mL |

**Intended use is for Research Use Only**

## Conclusions

**The CaptureSelect Lenti VSVG affinity matrix can be used for efficient purification of Lentivirus particles, pseudotyped with VSV-G. It is the first affinity chromatography resin available for Lentivirus purification, offering high recovery and purity in a single capture step, without compromising infectivity of the lentivirus particles.**

### Acknowledgements

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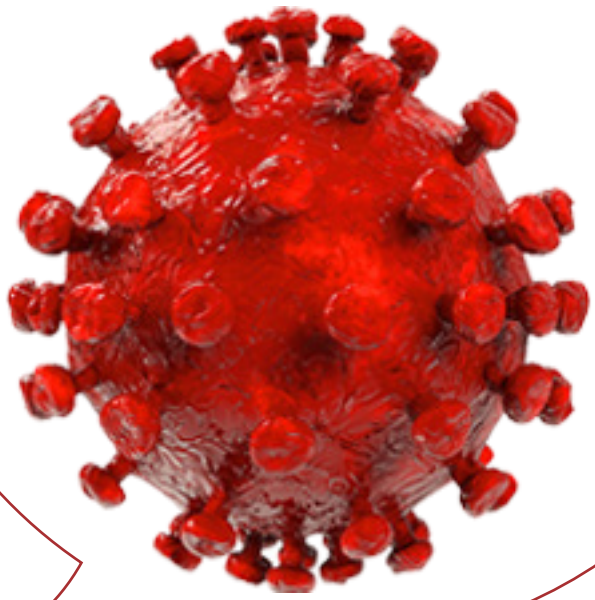
FAST FACTS

FAST FACTS VIDEO



# Efficient, scalable purification of VSV-G lentivirus by novel affinity chromatography

## Thermo Scientific™ CaptureSelect™ Lenti VSVG Affinity Matrix

Lentiviral vectors have emerged as a long-term stable gene expression tool for cell and gene therapies. However, large-scale production of purified clinical-grade lentiviral vectors remains a challenge because of the complex feedstock and its sensitivity to changes in temperature, ionic strength, pH, and other environmental factors. With these methods, general recoveries in the field are not higher than 25–30% for the overall process, with a significant part of the losses being in the final filtration step utilizing a sterilizing-grade filter.

In this **FAST FACTS VIDEO** Frank Detmers, Director of Ligand Application for CaptureSelect, Thermo Fisher Scientific, introduces a novel affinity chromatography resin—CaptureSelect™ Lenti VSVG Affinity Matrix—developed by Thermo Fisher Scientific as a solution to these challenges.

Features of this affinity matrix include:

- Efficient and high-yield single-step purification of lentivirus particles from recombinant suspension cultivated harvest
- Gentle, neutral pH elution conditions to retain lentiviral activity
- Excellent scalability
- Non-animal derived

CaptureSelect™ Lenti VSVG Affinity Matrix offers an efficient and scalable purification method for VSV-G pseudotyped lentivirus particles, with a binding capacity of $1 \times 10^{11}$ total particles/mL resin. Elution can be performed using gentle elution conditions, which helps to retain infectivity of the enveloped virus particles.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INNOVATOR INSIGHT

# Manufacturing considerations underpinning viral and non-viral platform selection

## Elisa Manzotti speaks to Allison Hagerman, Aaron Noyes, Vladimir Slepushkin & Laurens Sierkstra

**ALLISON HAGERMAN** is a Professional Engineer focused on biotechnology, Ms Hagerman joined Oncolytics in 2010 and has been integral to the progress of its product development program ever since. Prior to being appointed as Vice President of Product Development, Ms Hagerman was the Director, Manufacturing and Engineering from 2013–2017 and Project Manager from 2010–2013, during which time she led the process performance qualification for pelareorep drug substance. Ms Hagerman is a Professional Engineer (P.Eng., APEGA) and Project Management Professional (PMP, PMI). She holds a Master of Biomedical Technology (MBT) degree from the University of Calgary, and BSc degrees in both Chemical Engineering and Biological Sciences. She is an accomplished equestrian and spends her spare time on horseback.

**AARON NOYES** is a Vice President of Integrated Drug Substance Development at Codiak BioSciences where he leads a team focused on developing scalable production processes for exosomes and robust technology to load varied payloads into exosomes. At the start of his industrial career, Aaron worked at Millipore before joining Wyeth Biopharma/Pfizer Biotech for 12 years where he developed purification processes and focused on scale-up of biologics, including mAbs, recombinant proteins, ADCs, vaccines, cell therapies, and viral vectors. Aaron received a BS in Biochemistry from the University of Massachusetts at Amherst, a ME in Biotechnology Engineering from Tufts University and an Engineering Doctorate in Biochemical Engineering at University College London.

**VLADIMIR SLEPUSHKIN** is Global Head of Manufacturing at MedTherapy Biotechnology. He is leading all functions associated with manufacturing of CAR-T cells and viral vectors. Previously he was Executive Director of Vector Technology at Autolus Therapeutics, leading process development for manufacturing of lentivirus vectors in suspension cell culture, guiding assay development to support process development for lentiviral vectors, managing CMO for GMP vector production and T-cell processing. Before that, Dr Slepushkin was directing research vector core, and providing lentiviral, retroviral and AAV vectors for Kite Pharma. Vladimir proved successful in developing novel high-quality products by managing diverse technical groups and cross-functional teams, developing first-in-class clinical product from scratch, including facilities, equipment, manufacturing process, quality systems, regulatory CMC submissions and clinical trials design. He has proven expertise in technically understanding and leading the development and improvement of cell culture and purification processes, and operations and analytical methods, adhering to customer, regulatory, safety and environmental requirements and guidelines. Vladimir is experienced in identifying and resolving regulatory and manufacturing technical problems, as well as intellectual property assessment and licensing. He has authored 61 scientific papers in peer-reviewed journals and he's an author on 14 patents and patent applications.

**LAURENS SIERKSTRA** received his PhD in biotechnology in 1994 from the University of Utrecht after studying biology at the University of Leiden. He then joined Unilever as Project Manager and Unit Leader. In 2005, after the spinout of BAC BV from Unilever, he became CEO of BAC BV and set up the business in using single-domain antibodies for affinity purification, called CaptureSelect, which was sold in 2013 to Life Technologies. Since the acquisition by Thermo Fisher Scientific, he has been the business leader for the affinity purification business within the Bioproduction Division.

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With an ever-increasing range of viral and non-viral technologies available to advanced biotherapeutics developers, manufacturing considerations must play a key role in the decision-making process behind platform selection. These considerations include the current level of innovation in the bioprocessing toolkit and its corresponding capability to address the specific challenges facing individual technology areas.

In this article a panel of experts spanning the lentiviral vector (LVV), exosome, and oncolytic virus fields discuss the impact of manufacturing considerations on their respective platform selection and ongoing product/process development strategies, comparing the state of the art in enabling technology in each application area, and discussing related challenges, needs, and opportunities.

## Q Can you each briefly introduce us to your organization's current activities?

**VS:** MedTherapy is a startup company in the Boston area. The company is dedicated to providing services as a contract manufacturing organization (CMO) for the manufacturing of CAR T cells and lentiviral vectors.

Our main goal is to make manufacturing cost effective for people in developing countries. We consider the cost of goods when developing our manufacturing methods. One of the features that distinguishes us from many other CMOs is that our manufacturing facility is located in India, near New Delhi. This will allow us to lower prices due to a reduction in labor costs. We are still in the facility building phase, and starting next year we will be operational and looking for partners and clients.

**AN:** Codiak is a therapeutics company that started in Cambridge about 7 years ago, focused on demonstrating exosome therapeutics as effective immune-oncology drugs. Codiak's key technology is engineering exosomes to modify the tropism and deliver different payloads. We use these effective delivery vehicles in a way that takes advantage of the fact that they are immunologically silent.

We currently have three clinical candidates in or entering Phase 1 trials. One of them has an engineered surface Il-12 cytokine that allows for engagement with receptors on NK and T cells. Another takes advantage of the synthetic payload synthesis route for a selective cyclic dinucleotide STING agonist and combines that small molecule with an exosome to enable selective uptake in tumor-resident antigen presenting cells. Finally, we are working with anti-sense oligonucleotides attached to exosomes to downregulate various transcription factors in hepatocellular carcinoma. Other constructs in the pipeline include encapsulating AAV to enable re-dosing, and using exosomes with combinatorial ligands to enable vaccines.

**LS:** The Bioproduction division of Thermo Fisher Scientific is the global leading supplier of both upstream and downstream consumables, hardware, and single use products used in bioprocessing. My specific area is in affinity purification. We enable customers working on new therapeutics to come up with platform processes for purification, which will result in safe, affordable products.

Our pipeline is usually composed of all kinds of new modalities, including ongoing R&D programs to support lentiviral or exosome purification. Our main focus is for our customers to receive good platform purification solutions and associated analytics to be able to scale up their process in an affordable way.

**AH:** Oncolytics Biotech is working on a cancer therapeutic using a non-pathogenic virus, with the active ingredient being the double-stranded RNA virus itself.

That product is currently in Phase 2 trials in a number of oncology indications, most notably breast cancer. The team I lead is responsible for the process development, manufacture, analytical testing and clinical supply for that product.

**Q** Can you tell us about the key manufacturing-related considerations that impact strategic decision-making around initial platform selection and subsequent early development activities in your respective fields?

**AN:** The first thing we focused on for exosomes, which are a new modality, was reducing the risk. This means first reducing the risk of supply. We needed to have technology that can be scaled up to GMP manufacturing in a predictable way.

As a small company starting out, we did not have our own manufacturing facility, so we had to use a CMO. As we chose our CMO, it was important they already had equipment that we needed and trained operators already familiar with technology, in order to de-risk the production process.

The other key strategic piece pertains to regulatory risk. As we looked at exosomes, we wanted to make the process more acceptable to regulatory agencies, which involved taking steps such as using well-known cell lines, avoiding animal-derived components, and crafting release and characterization assays that build on the established state-of-the-art for recombinant protein production.

**LS:** For these early technologies, adding new components can complicate things, so de-risking those aspects is key. However, it can be good to utilize new technologies to deliver short-term improvements. That is where we sometimes help customers with their challenges, for example in purification, and we work with them to deliver a scalable solution.

**VS:** There are two viral vector platforms that can be used for CAR T cell transduction, either retroviral or lentiviral. The difference between these two platforms is connected to manufacturing in various aspects.

Lentiviral vectors are easy to make with relatively high titers for transient transfection, making it a very common platform. However, it can be difficult to make a stable cell line that would produce these vectors, which can limit large-scale manufacturing.

Retroviral vectors are made mostly using stable cell lines, making large-scale manufacturing easier. However, you cannot create high titer vectors with transient transfection for retroviral vectors. For early-stage development with varying vector design, it can be difficult as you need to make a stable cell line each time.

**Q** How would you sum up the current status of the bioprocessing toolkit in your respective fields? What have been the important recent advances, and also the important innovation needs?

**AH:** Oncolytics' lead product is relatively simple by today's standards of viral and immuno-therapeutics. The toolkit we have available to us is fairly extensive, from old standbys to newer technology with more advanced controls for improved yields and outcomes.

From my perspective, the biggest recent advance is the widespread availability of single-use systems. This not only means single-use reactors, but also prepacked columns and single flow pass disposable items. These are appealing to CMOs because it limits their workload and provides ease of switching for varying client processes. If all these things are single-use systems, they tend to be accessible in a variety of locations at different facilities. This makes the process more flexible and adaptable for extension to other markets down the road.

In my experience, the most important challenge is limited facilities for live virus production. There is less innovation in the near-term in this area.

**LS:** We work on many different modalities at Thermo Fisher and every modality comes with its own challenges. For example, monoclonal antibodies have a different innovation need and drive than oncolytic viruses. Another example is lentivirus, as only a small subset of the material you produce can infect cells.

We work with companies that know their specific application, molecule, and challenges, to make those step changes in productivity that are often desperately needed.

**AN:** I echo the point that single-use disposable components are a key part of how we operate. For exosomes in particular, re-uptake of vesicles is a phenomenon by producer cells. Perfusion cell culture, wherein the released exosomes are rapidly separated from the cells, has been effective in terms of increasing titer. It however has introduced the challenge of separating 200 nm bio-nanoparticles from cells that are several µm in diameter. There is a need to grow the technology to do this.

More broadly we need to reach a critical mass in the industry to help drive innovation, learn from leaders, and enable enough large companies to share what they are doing in order to build into each other's advantage and thereby help the entire sector flourish.

**Q** For your specific class of molecules, what is the biggest challenge you see for achieving commercial production scales, specifically – or if production-scale has already been achieved, what would be the biggest improvements that would add the greatest amount of value to the manufacturing process?

**LS:** In this area, we have always been working on different new modalities and every modality has it specific unique requirements. For example, AAV started around 15–20 years ago, with people wondering what the platform system was going to be in terms of serotypes. The biggest step change was starting to make products for single serotype forms, to enable a scalable system. Later on, we launched a product that could do all serotypes as opposed to only one, which became the platform for AAV manufacturing independent upon serotype.

As a technology supplier, we moved towards focusing on what different types of modalities are being chosen. From our point of view, zooming in on the platforms that people will be adopting is one of the biggest challenges. Resolving this enables products which can be used to support that platform. The key step change is going from a plethora of different technologies,

for example to purify products, to real platforms which can do that, which then streamlines and the use of a specific technology in early research into process development and finally into manufacturing for all new therapeutics derived from new modality platform.

**AH:** Scalability is a product of your process but also your materials. In addition to scaling up and out for larger volume production, we are also scaling up and out for later phase production, and eventually commercial production. This needs to be factored into planning as early as possible with your manufacturing partner, to avoid a situation where you are using reagents and materials that are suitable for early phase and not later phase.

Use of non-animal component-derived material and sourcing of materials that are fully cGMP suitable for later phase production will avoid the need for comparability efforts either in the clinic or in the manufacturing pipeline. Imposing or at least developing reagent specifications and controls early can help with scalability later.

**VS:** The greatest advantage for production at commercial scale is the development of stable cell lines for lentiviral vectors. Both lentiviral and AAV vectors are mostly manufactured by transient transfection. The main disadvantage of transient transfection is the need for a lot of plasmid DNA and expensive transfection reagents. Creating a stable cell line that does not require plasmid DNA to make vectors greatly improves scalability of the process.

The problem with this stable cell line is that some of the vector components are toxic to the cells, so you need to regulate expression. So far, several systems have been used with some success, but for lentiviral vectors, we are not yet ready to use this platform for commercial manufacturing.

**AN:** From my vantage point in exosome production, we run 500 L perfusion reactors, turning over a bioreactor volume a day. Over 20 days, we produce 10,000 L.

The production scale we are at, combined with the reasonable likelihood of doubling that scale, gives us ample material for commercial supply. The challenge now is ensuring consistency and safety of the product. One of the challenges in the bio-nanoparticle space is ensuring virus and adventitious agent safety. If there were inactivation technologies that were suitable for use with enveloped particles, that would be a huge advantage. That is one area we need advancements.

The other point speaks to comparability. The more complicated and the newer the modality gets, and the newer it is, the less understanding you start with. For the larger bio-nanoparticles, especially when they are relatively early in clinical progression, there is not a full sense of all the critical quality attributes (CQAs). It is important to define the likely CQAs early on and make the effort as a community to ensure CQAs are well understood to ensure comparability throughout process changes and batch.

**Q** What are the biggest challenges relating to the current toolkit – in particular, its scalability for commercial production when considering downstream processing and analytics?

**AH:** In my experience, which is limited to production of an infectious viral vector itself, the downstream processing scalability has been relatively straightforward.

There is certainly room for optimization, simplification, and improvement, but we are comfortable with the accessibility of the technology itself.

The more interesting challenge for our product type falls in the analytics. In any given situation, the variability in production is no higher than the variability in the analytical testing. There is room for improvement in understanding that range, the appropriateness of those ranges, and possibly looking to custom methods and consistency of outcomes. There is room for different manufacturers and analytical labs to support those types of activities to help these new molecules progress through the development pathway. The sooner we can start exploring those, the better.

**VS:** I agree that analytics present a more complex issue than downstream processing for lentiviral vectors. Downstream processing is relatively established with combinations of chromatography and tangential flow filtration (TFF). The only challenge in downstream processing is formulating the vector to avoid aggregation.

In terms of analytics, there are many challenges, for example variability in the titer determination. There is also no standard in the field that allows comparison of titer results between different companies. The results are often dependent on how the assay is performed. Another challenge is developing the potency assay, which can be difficult for these vectors, because they are used as an intermediate material to transduce T cells.

A third analytical challenge is the replication-competent lentivirus assay, as this assay is complicated, time-consuming, and expensive. In my view, it is not necessary, but the FDA and European agency still require this assay. It creates additional hardship and raises the price of the final product.

**LS:** The analytics bottleneck certainly applies when working on something relatively new. As a technology provider we always like to get into contact with customers who have specific issues, because there are many tools available within our company to help with these developments.

An example, which is close to our own purification products, is that when we develop a purification resin, those same ligands can be used for quantification and titer analysis, usually on any commercial analytical platform. Each analytical challenge can be overcome using the right tools.

**Q** Looking to the future, what would be the next-generation technologies for your specific platform areas that would represent a breakthrough?

**VS:** In terms of CAR T cells as a cell therapy product, next-generation technologies are being developed to shorten the time of manufacturing. Now, the time to manufacture T cells is between 7 and 10 days, and considering the time to test and release cells, it often takes about a month from needle-to-needle for this product. That is challenging both in terms of pricing and for the patients, so it is critical to decrease it.

There are also developments in making allogenic CAR T cell products. Some companies are even trying to make lentiviral vectors that could be injected directly into patients without the necessity of making CAR T cells *ex vivo*. If this is successfully developed, it could be a huge advantage for the future.

**AN:** Single particle analysis would be a breakthrough, similar to how fluorescence-activated cell sorting has developed understanding of cell biology, allowing sorting for different markers. For exosomes, and this class of ~100 nm bio-nano particles, single-particle characterization would enable an understanding of the population you have and potentially enable the development of surrogates for potency. If you could subsequently sort those, that would be incredibly valuable because you could then directly connect potency to phenotypic properties of the particles.

**AH:** I agree with what's been said so far, and it touches on this concept of timeliness of information related to your production process. The quicker we can have readouts of the state of the process, whether that is the state of cell expansion, the infection process, or other elements depending on the molecule up for discussion, the quicker the overall production. Many of the analytical methods available at present are robust but time consuming, and only allow data gathering after a batch has completed.

The ability to get readouts mid-process that could be applied to decision making would provide an opportunity to optimize these biologics productions at exactly the right moment. For me, technology in this area that would be the most interesting development.

**LS:** In the purification and downstream area, one of the real breakthroughs would be if one could remove packed bed column technology. The volumes associated with new modalities are quite different than those in, for example, monoclonals or recombinant proteins, so that could make the area more amendable to this.

For example some new modalities could lend themselves to magnetic bead approaches like in cell therapy, where you move away from traditional purification steps.

**Q** What would be the key advances in innovation specifically for downstream processing and analytics?

**AN:** Robust particle sorting is technologically beyond the current technology. Affinity chromatography is a way to complement analytical characterization by sub-fractionating exosomes. Fusion of analytical techniques with the purification technology could be useful to drive potency higher or amplify selected properties.

Another unknown in analytics is what makes a potent particle. In viral vectors, where the potency per particle is relatively low, it is not always understood why this is. In exosomes, the reason behind the potency of the particles is also often unknown. Developing the characterization technology to enable that would be powerful.

**VS:** One of the most important things in the lentiviral vector field is developing lentiviral vector standards that everyone can use in their lab, and qualify it there, using a titer assay.

**Q** What are the keys to successful collaboration between end users and bioprocess solution developers/providers, with a view to getting these new solutions introduced into processes?

**AH:** The key to any successful collaboration is clear communication. This means a clear understanding of both the requirements of the clients and the services available from the supplier.

Transparency around any roadblocks or bottlenecks leads to an efficient, smooth collaboration. If there is a circumstance where a group either is unfamiliar or uninterested in a certain scope of work, we can source the right partner for that collaboration and potentially pull teams together where possible. More generally, early conversations between stakeholders considering scope and scale allow providers to clearly see market needs and ensure they are solving a valuable unmet need.

**LS:** From our side, the focus is on openness and clarity. We have always been successful in working with customers to develop new areas. Our current product for AAV was fully developed together with a company in France and is now being used by most people working with and purifying AAV.

Collaboration requires openness, willingness, and the realization that certain advances will help the whole field. With these factors, there is no limit in terms of the products and areas that can be developed.

**AN:** Openness between companies like my own and vendors is happening. You need to have that trust to make breakthroughs. It has still been difficult to share externally, although this is changing as companies gain more confidence, and as technology advances. In the AAV field, the amount of collaboration is tremendous.

A successful collaboration requires openness and trust that allows you to use authentic materials, and transfer between sites. It is also important to build in time for iteration.

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## AUTHORSHIP & CONFLICT OF INTEREST

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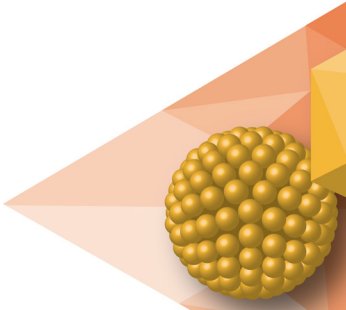


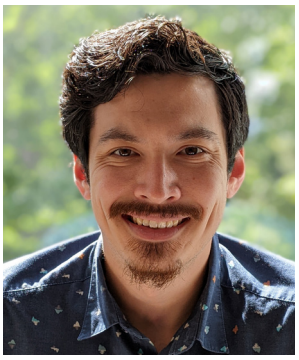
CHANNEL CONTENT

DOWNSTREAM BIOPROCESSING

INNOVATOR INSIGHT

# Addressing current challenges in lentiviral vector purification & associated analytics

**Charlotte Barker**, Editor, BioInsights, talks to **Bryan Zee**, Associate scientist, Juno Therapeutics and **Anindya Dasgupta**, Director of GMP, EXPRESSION Therapeutics



**BRYAN ZEE** started his purification career at Amgen's PD group where he developed several clinical stage biologics and handled a myriad of modalities such as mAbs, bispecifics, and Fc-fusion proteins. Since 2019 he's been at BMS's viral vector PD department where he has developed BMS's LVV purification platform and AAV purification platform.

**ANINDYA DASGUPTA** is the director of vector development at Expression Therapeutics. He obtained his PhD from University of South Carolina, USA. His post-doctoral training and research associateship at the school of medicine, Emory University, Atlanta, USA, were focussed on the evaluation of novel anti-cancer therapies and the development of strategies for expansion and lentivirus based bioengineering of γδ T cells in serum free media . Anindya is a co-inventor of a patent on anti-cancer strategy. At his recent role at Cincinnati Children's Hospital Medical Centre he led vector development to manufacture high titer lentiviral vectors.

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**Q** How would you characterize the current state of the art in lentiviral vector purification? Where specifically are you seeing progress in improving vector recovery and quality?

**BZ:** 'State of the art' means different things depending on the stage of the program. Early-stage clinical state of the art is suspension-based feed stream, a centrifugation-free clarification, chromatography capture, tangential flow filtration (TFF) concentration, and sterile filtration. As we move towards a late stage or commercial process, we are transitioning away from these early academic-style processes into the 'classical bioprocessing style'. Specifically, I am seeing some encouraging progress in understanding how lentiviruses (LV) are reacting to chromatography-based capture as well as sterile filtration, with improvements in recoveries. As far as quality, we are starting to move on from a titer-based method toward looking at other quality factors of these vectors, such as if they have sufficient pseudotyping. There is still a lot of work to be done to move away from molecular biology-based to first principal style measurements and move towards a good manufacturing practice (GMP) commercial setting.

**AD:** In terms of the perspective of a GMP manufacturer, you can have an adherent-based or suspension-based system, which means you need to be fully aware of your clarification, purification, and polishing steps. You must select a process that you can bring to GMP, which may not be possible or prudent in the earlier phases. Keep in mind that if it works early, it does not mean it will work at the GMP phase. You will save a huge amount of time and money if this is considered early.

Secondly, you need to have more closed processes. There are closed methods that you can replace your open manipulation with, such as acoustic-based cell suppression systems as an alternative to centrifugation-based methods. There are also efforts for the clearance of contaminants and residual plasmids using endonucleases that are active at high salt concentrations to elute the vector from chromatography columns. There are also developments that can be brought into GMP phases in terms of monolithic chromatography.

Thirdly, you need to know the quality of your particle quickly. Virus analytics platforms are crucial because cell-based systems can take a week to deliver an answer which is required in a few minutes. You need to adapt to that platform technology as soon as you can.

**Q** What do you see as the must-haves for downstream processing in terms of knowledge of the upstream lentiviral process?

**AD:** Optimization by quality by design methods is important. You can run multiple small bioreactors to get things done quickly. Process knowledge, such as design and control space criteria for your upstream method, is important. Recovery including quantity and quality is important. Keep in mind that at the early stage, your requirements will be substantially different than at the late phase investigational new drug (IND) stages . You need to be adaptable and flexible to differing needs.

**BZ:** You should have an intimate knowledge of your upstream process as well as its development. I would personally characterize lentivirus upstream production as 'weird'; there are many things we do not understand in that actual transient transfection process. They can have a significant effect on downstream, so having a good understanding of where your vector feedstock is coming from will help you develop your own downstream process. Discovery is different to IND and IND is different to Phase 2 process characterization. The more adaptable you are in terms of understanding your upstream process, the more you will understand how your downstream process reacts to your upstream process. This will prepare you for more successful process characterization instead of simply doing the bare minimum to enable an IND and then waiting until Phase 2 to do the process characterization.

If I had to give my upstream team a list of information I needed from them, I would include transfection details, including the transient transfection method and titer consistency, and the production method used, whether adherent, suspension, or microcarrier cell culture.

**Q** Regarding scalability, what are the current challenges in LV processing stages? What repercussions are there for downstream processing?

**AD:** Regarding scalability, what you start with at the beginning of the process is important. This is most likely an adherent-based system, which works fine, but can only be scaled out rather than scaled up. This option is labor and cost-intensive, and can lead to batch-to-batch variation. However, there are large advancements in this field, such as the fixed bioreactors from Pall and Corning, that are enabling scale-up for adherent systems. We do not want to rule out adherent as a future scalable approach. However, one must keep in mind that you need to run these fixed-bed reactors in parallel. This requires retrofitting existing infrastructure or building new systems. Many vendors are more than willing to help you in designing your space to accommodate what you need to achieve. Adherent-based systems could be a future scalable approach, though they do need to be optimized.

As a company, we are pursuing a fixed-bed bioreactor platform for our internal products and with an option to offer that to external clients. We have process development (PD) and GMP manufacturing all in the same building, so whatever we do in PD, work needs

to happen in the GMP space as well. If you started with an adherent system, there is a significant investment in terms of time, people, and cost required to adapt the system to a suspension system.

However, if you want to be the first to market for a disorder, you are perfectly fine with a small setup. For personalized medicine, you do not need a suspension system because you are only treating a few patients for a rare disorder. There are issues all the way across the product life cycle that you need to be aware of when you scale from one level to the other.

**BZ:** The last downstream unscalable step that we had was ultracentrifugation, which has been phased out quickly. The last real bottleneck for scalability is the culture method. You can brute force the adherent scale-out methodology – I have seen batch sizes of 36 HYPERStacks with a 200 L total harvest volume – but it is tough to run at that level in terms of GMP. You must know what you are doing in order to lock in the reproducibility of that style.

In my opinion, we need to move to suspension to enable scale-up. However, when you move to a suspension from an adherent production, there are some significant implications for your downstream process, such as the need to separate cells, alter clarification steps, and deal with significantly increased biomass load. In addition, the need to change cell lines alters the impurity levels. Switching to suspension can lead to higher or lower host cell proteins.

**Q** If producer cell lines for lentivirus become state of the art, how would downstream processing need to adapt accordingly?

**BZ:** Producer cell lines are one of the great white whales of lentivirus. They are a tricky thing to pull off because lentivirus components are inherently cytotoxic to the cells, which means that those components need to be inducible rather than constantly expressed. This raises the question: are cell line scientists going to figure out how to keep a continuous cell culture going with a slow, diluted secretion of lentivirus? Or will inducing cells to produce lentivirus require an increase in cell mass, to make up for the cell culture death? The downstream needs to remain nimble when it comes to producer cell line possibilities because both ends of the spectrum have different requirements: when dealing with a dilute stream, your primary problem is volume concentration and managing large volumes on the commercial floor; when dealing with a highly concentrated, highly impure feed stream, you need to figure out how to clean up without losing too much vector.

**AD:** Producer cell lines started with gamma retroviruses, and people saw the adverse reactions that can happen from these. They were the first to make constitutive cell lines a few years ago, which is still ongoing, but vesicular stomatitis virus G (VSV-G) may be a big culprit in terms of toxicity, for example. However, there are systems, such as the LentiPro26 system, that are engineered to overcome toxicities.


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You can also have inducible cell lines using antibiotics, which require complete removal of antibiotic traces from the final product. From the GMP manufacturing point of view, your release test is all that matters. It should not detect whatever is unintended in your final product.

One advantage of producer lines is that you do not need plasmid DNA. There is currently a big bottleneck in acquiring large volumes of plasmid DNA, especially for GMP grade.

It is important for upstream and downstream to communicate. You might be harvesting in a continuous manner for a few days or weeks, but your downstream processing may only be adapted for two-day cycles. Lentiviruses are not that stable at room temperature long-term, so you need to find ways to capture those harvests and maintain them, which can be difficult with continuous processing. A producer cell line is great, but one should be cognizant that you will need to make substantial changes along your product manufacturing timeline. The future might be cell-free vector production.

> "It is necessary to develop early evaluation workflows to have a more holistic development effort, so that if the upstream team finds a good condition, then you can evaluate that at an early stage and determine if it is actually useful."
> - Bryan Zee

**Q** What could be done in downstream processing to address challenges stemming from upstream, and what is your key advice in this regard?

**AD:** All the upstream adaptations are extremely important to the downstream stage. You need to have a good understanding of where you want to be, so you can develop your PD stages accordingly. As you move upscale, remember that every vector product is unique, so you need to have ongoing continuous engagement with the respective departments along the life cycle of your product. In Phase 1 your requirements are similar to those at the IND stages, but as you move to a larger scale, you need to be aware of what needs to happen in Phase 3 and beyond. This is not only restricted to bioprocessing; it will also affect your bioanalytics portion, and this can influence your downstream processing and how much purification is required.

**BZ:** My advice would be to work closely with your upstream team. The things that your upstream team will do to enhance their cell culture and titer have a high probability of affecting downstream behavior. If you are not in good communication with your upstream team, the changes that they make to increase their titer and culture performance might end up

being a net negative because they affect your downstream process to a large degree. It is necessary to develop early evaluation workflows to have a more holistic development effort, so that if the upstream team finds a good condition, then you can evaluate that at an early stage and determine if it is actually useful.

**AD:** End-of-production cell analysis is a process that must be done at the end of manufacturing. You must harvest your cells and submit them for release testing and quality control testing. Conversations with upstream people are important because they may identify a system, but that system may not allow you to harvest the cells at the end. That is a common problem for fixed-bed bioreactors. Keep in mind that you will need to harvest some of those cells at the end, and get your upstream to support that.

**Q** Where in the lentivirus process are bespoke solutions to the field most needed?

**BZ:** Filters and chromatography solutions are where bespoke solutions are most needed. I'm a chromatography nerd at heart, and an affinity ligand would go a long way towards adding to the overall robustness of lentivirus production. When using a non-affinity modality as the capture method, you are more sensitive to variation in cell culture. If you move to protein A affinity-style capture, it can expand the robustness of your downstream in order to absorb more variation from the cell culture realm. The lentivirus is a very sensitive vector – to both salt and pH – so it is no small feat to get a functioning affinity ligand that is ready for the GMP primetime.

Lentivirus clarification, where lentiviruses are big, heavily charged particles requires a new kind of filter. The classic depth filter styles do not necessarily always work well with the charged nature of lentiviruses that. At the same time, Sterile filtration lentivirus is approaching close to 0.22 µm pore size. Running lentivirus through a sterile filter is no easy feat, so a more bespoke manufacturing style of a sterile filture tuned for filtering something large like a lentivirus would be helpful.

**AD:** One also needs to be aware of the optimization of cryopreservation formulations because as your vector production lots get larger, you need more data in terms of stability and formulation. With increasing volume and scale, you need to investigate time versus stability. You may have to optimize your cryopreservation formulation to accommodate these highly complex particles. Improvements in the freezing process are also to be taken care of as well.

**Q** What are the current major challenges and shortfalls on the analytical side of LV manufacture?

"Even though we have been using lentivirus for some time now, there is extremely limited information available on the vector particles themselves. Characterization is very important as you develop your analytical assays."
- Anindya Dasgupta

**AD:** Even though we have been using lentivirus for some time now, there is extremely limited information available on the vector particles themselves. Characterization is very important as you develop your analytical assays. Unlike for AAV, we do not have universally accepted reference material to base analytics on.

I am currently setting up our quality control and this requires a reference to ensure that the assays and operator are being qualified. There are efforts towards this, but those references are not universal.

We are still dependent on a system of 293T cells, which are easy to transduce. We need to come up with a method where we can transduce the target cell of interest. Taking the titer data from 293T cells and applying that to bioengineer your cells of interest is a method that needs improvement.

Virus analytics are important and they need to be purpose-built to determine quality rapidly and accurately. The technology is already out there for this, using light scattering and Brownian motion.

Lastly, we need to come up with better product characterization methods that are unique to viral vectors. Many methods used now are carried over from the antibody production world. Lentivirus is unique, so we need to come up with new and novel methods.

**BZ:** One of the major challenges is the turnaround time for these cell-based assays. Cell-based infectious assays can give false trends if the variables are not tightly controlled. It can be a challenge to establish a viable scale-down model for your therapy.

Biophysical cell characterization is starting to develop, but I have yet to see it fully ready for the primetime qualified GMP stage. Multi-angle light scattering has a lot of promise for looking at lentivirus, but it still needs more work to move onto the qualified GMP stage. The potency question is the number one challenge – we still need to understand what makes a vector effective. We need to determine our critical quality attributes and find out how to make these particles more effective. The typical antibody mentality of 'aggregates equal bad' might not hold true with vectors. We need to have the assay panel to be able to find that out and make educated decisions around these attributes.

**Q** What new analytical methods could help us understand the 'weirdness' of lentiviral production?

**BZ:** A robust scale-down model can help you find an experimental lever that you did not previously realize was a factor. There are many interesting imaging assays with the potential to help us understand how a vector comes off a column. There are a couple of assays where a sophisticated camera tracks a particle as it vibrates through space which helps us count. Also, you can always go back to the classics, such as confocal microscopy. We need to remember that lentiviruses are bags of fluid, they are not proteins, so they behave more like liposomes than monoclonal antibodies.

**Q** Could you each pick out three key priorities for future research and innovation in lentiviral processing, and where do you think investment is most needed?

**AD:** First, a producer cell line, or at least a packaging cell line, is important, to improve batch-to-batch consistency. Second, the loss of lentivirus in the downstream can be significant; recovery of 25–40% in the current state of manufacturing is all you can expect. We need to have disruptive advancements in this field, meaning we need different purification columns. We cannot depend on elution with 0.5 monosodium chloride anymore.

Analytical developments are very important. Since these technologies have been coming in, regulatory agencies are cognizant of the fact that you can take advantage of these methods to characterize your virus. They are going to ask you for that data, and how much you know about your drug product.

Finally, we need to substantially bring down the cost of goods. Currently, the drug price is in the range of a few million dollars, which can be incredibly difficult to get to patients. Having single-use technologies needs to be improved upon, for example, can we integrate the clarification step with the upstream step? Can it be integrated rather than harvesting? Can the harvesting be done coming out of your upstream directly into your clarification step? These things might help alleviate some of the costs in the manufacturing part so that the drug price stays low.

**BZ:** Cleanable affinity chromatography would go a considerable way towards enhancing downstream robustness. It is not that useful to have a column that does great at cleaning things up if it only works a single time. It needs to be cleanable using typical standards.

One of the key priorities is understanding the molecular biology around lentivirus production. We are taking two highly conserved biological processes and combining them. We need to understand on a fundamental level how these two systems work in order to increase the overall productivity of these vectors within cell hosts.

Sterile filtration in lentivirus purification is usually a step that can cause the loss of up to 90% of your product. If the step is properly optimized, you can expect 50–70% recovery, which is very low compared to monoclonal antibody filtration.

## Q Do you measure infectivity titers during PD stages? And at what stages during manufacture are you testing productivity?

**AD:** You have to keep infectivity in mind at all stages of development. Some transients are notoriously difficult to express and give a low titer. As you scale-up, be aware that your titers may decrease a little, so you must produce more to get the number of vector particles you need at the end.

**BZ:** You should measure infectivity titers during PD stages as often as possible, provided your infectivity assay has the throughput available to power your studies. Cell-based assays do not usually have that much throughput, so you may need to make a trade-off for a higher throughput method. You can measure the RNA genomes of the particle, but that is not the actual infectivity portion of the vector. In an ideal world, I would have a magic assay to which I could submit hundreds of samples and it would give out an actual infectivity titer. Oftentimes, to do effective, time-efficient studies, you need to make trade-offs between the assays to look at during PD.

## Q Lentivirus is temperature-sensitive, so how do you keep the fast protein liquid chromatography (FPLC) steps shorter or at a lower temperature?

**BZ:** Lentivirus is temperature-sensitive, but before you start specifically designing your chromatography step with this stability in mind, verify that it is as sensitive as you think. I have seen lentivirus that will completely lose infectivity at room temperature, but I have also seen lentivirus that can hang out at room temperature perfectly fine at varying levels of salt. We still do not fully understand what causes the sensitivity.

If your lentivirus is temperature-sensitive, there are a few ways that you can keep the FPLC step shorter. Convective-style chromatography is a fast way to work with vectors, such as with monolithic membranes. Those cycle times usually are on sub-hour cycles. It is a great way to get the vector on and off the column quickly. To control temperatures, at small-scale, use fridges to maintain low temperatures, but at large-scale, a CMO will need chillers for their mixing vessels and column. This is where things start to get tricky.

## Q As more companies are looking to use lentiviral vector for direct *in vivo* injection gene therapy, what will downstream processing look like in those cases?

**AD:** The first thing that comes to my mind is the safety of the product. You need to have a system that is more streamlined, with the least number of open manipulations.

In the second phase, there are various aspects to this, such as handling and stability during harvest and purification. You may have to adjust your downstream processing to fit that and meet regulatory expectations. You need to be cognizant of and familiar with the FDA requirements surrounding handling, storage, and release testing. This all comes into play for direct *in vivo* injections.

## Q What are the safety considerations for producer cell lines?

**AD:** We do not have a good processor line yet, but looking at the last few years, no lentiviral-based therapy has proven to be unsafe. All of the adverse reactions that were noticed in lentiviral-based therapies do not point to the lentivirus particle itself, but rather to its molecular design, such as the promoter or the affected cells. Lentivirus is split into various plasmids to nullify the *in vivo* recombination events, and as of now, there is no concern in the recent findings that it is unsafe. I do not think a producer cell line would be any more unsafe than what is being currently used in terms of transient transfection. The evidence suggests both would be safe.

**BZ:** In lentivirus production, the main concern is replication competent lentiviruses which caused an initial split of plasmids, but there has been a lot of work around evolving that bit out. I do not think there is much of a safety concern around this particular aspect.

## Q How do you deal with residential DNA as a contaminant?

**BZ:** Residual DNA is a fairly common contaminant to contend with. The current method is a nuclease-based digestion, which chops up the individual DNA into various small base pairs to reduce the risk of it as a contaminant. However, it would be more ideal to simply remove the DNA, which for chromatography requires a finer polishing step. Lentivirus is similarly charged to DNA, so you have to screen a fairly sophisticated polishing step, such as anion exchange chromatography or multi-modal chromatography, to find a condition where your lentivirus and your residual DNA are resolved out. Then, you can wash out your DNA or simply leave it bound on the column while you elute off your lentivirus.

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INNOVATOR INSIGHT

# Removing technological barriers to efficient large-scale LV vector production

## Charlotte Barker speaks to Scott Jeffers, Emily Jackson-Holmes, Rakel Lopez de Maturana, Steve Milian & Margherita Neri

**SCOTT JEFFERS** is the Chief Technology Officer at Gensight Biologics. He has worked with numerous biotech and pharma companies to bring novel gene therapies to the clinic both at clinical and commercial stages, for rare monogenetic diseases. Scott leads teams that utilize novel techniques and approaches including single-use suspension and adherent cell bioreactors, and adherent cell culture in Hyperstacks to produce viral vectors, such as AAV, adenovirus, lentivirus, retrovirus, and herpes virus.

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**MARGHERITA NERI** is the Vector Process Development Manager at AGC Biologics. She has considerable knowledge and experience in the design of vector production processes and at AGC she is involved in the process development and industrialization of large-scale production of lentiviral and AAV vectors in compliance with GMP requirements.

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As demand for lentiviral (LV) vectors for both *ex vivo* cell-based and *in vivo* gene therapy applications grows, the question of how to make LV vector processing faster, more productive, and more cost-effective becomes increasingly pressing. In this panel discussion, LV processing and quality experts from across the biotech, CDMO, and solution provider sectors will discuss how recent technological innovations in specific upstream and downstream LV process steps compare in terms of their impact on titer, process speed, and cost.

**Q** What can you share about your own experiences of seeking higher LV titers and improved process speed and cost through your choice of bioreactor and upstream production platform?

**EJ:** When developing products for upstream LV production, we use suspension-based systems, because they are advantageous in terms of scale-up, in addition to reducing variability and cost. To increase titer within the suspension system, we have used design of experiment (DoE) to optimize concentrations and timings of each component in the process, including the mammalian cell transfection process, cell line, transfection reagent, plasmid DNA, and any enhancers or supplements. This has resulted in a successful, optimized system that significantly increases titer and reduces cost.

**MN:** At AGC, the priority for our platform is to have good scalability between the full-scale and the small-scale processes. We chose Bioreactors for scale-up that have a fully representative scale-down model system. We have experience in scaling up to 200 L, with any major challenges being resolved at the small scale by applying DoE for all critical steps.

**RL:** From my experience, there are critical process parameters and key steps in production that we can optimize. For example, we can increase titers simply by changing the producer saline.

## Q What recent technological innovations are having the greatest impact on downstream LV processing?

**SJ:** It is important to understand the full process and process steps to best gain efficiency. If you have a large-scale production in your suspension system, you have a large volume on the first-capture step and the filtration steps, and every step in between. Using DoE to understand your steps, inputs, and outputs is important. Analytics are also highly important in having tight control over your outputs.

**MN:** The downstream side for LV is challenging, particularly because of the ~0.1µ dimensions of the LV. The most critical step is the final sterile filtration, where a large part of vector preparation is often lost. Clients frequently ask for more concentrated vectors. From a CDMO perspective, we must balance the concentration with the yield of the final sterile filtration. The more concentrated the vector, the more aggregation in the vector preparation, and the greater difficulty in balancing sterile filtration. Improvement in the analytical possibility to evaluate the vector aggregation will be important to solve the downstream challenge. Recently, many new membranes and resins for purification have become available on the market, and we are testing these to improve LV purification.

**RL:** There have also been advances in the fields of affinity, size-exclusion, and ion-exchange chromatography. Quick analytics that enable definition of critical process controls and use small-sample volumes are key. Automation in fill-and-finish and other processes allows better stability.

## Q What scales have you reached for suspension? Either for transfection or stable cell line?

**EJ:** For transient transfection suspension, we have reached 50 L in-house, but we do have customers reaching as high as 200 L with our products. When dealing with transfection at that scale, we have optimized different aspects, including the timing of the complexation, keeping the reagent cold, and the mechanics of adding the complex to the bioreactor.

## Q What analytical tools are helping you to improve the identification and measurement of critical quality attributes (CQAs)?

**SM:** Some of the common tools that are currently deployed include digital PCR (dPCR), quantitative PCR (qPCR), P24 assays which are typically ELISA-based, and flow cytometry. Without a doubt, dPCR and qPCR are the bread and butter for LV analytics, giving several CQAs such as genome titer, infectious titer, and residual DNA. These methods can also provide us some insight into the average number of genes of interest (GOIs) that have integrated into a cell line, which is an important CQA.

Genome titering has been challenging in the past, mostly because of residual levels of the genome of interest (GOI) that can lead to an overestimation. Strategies to overcome this include optimizing the endonuclease step to remove residual DNA. This is useful for quick turnaround results, compared to the cell-based infectious unit (IU) titer methods.

There are several commercially available kits for P24. They bring some challenges, such as a lack of differentiation between free P24 and particle-associated P24. This is an important consideration, as certain processing steps can damage the virus and give erroneous titers. Commercial vendors are working on this, but there is certainly some room for improvement.

Lastly, flow cytometry is another important method, mostly used in potency assays. Some of the challenges in flow cytometry revolve around data analysis and ensuring proper gating parameters.

**MN:** Regarding analytics, in our CDMO we have a strong interaction between process and analytical development. Together, we define the main parameters that we want to monitor in our process. According to these, we decide the best method for each step. Each step is always monitored, with orthogonal assays for each CQA. For LV vectors, the main test for potency evaluation is infectious viral titer. The analytical method for this assay must be robust, reliable, and reproducible across labs, in order to control the consistency of the vector production processes. If the vector is applied for *ex vivo* gene therapy, we need to use a cell line for infectious viral titer evaluation that is transducible with the same efficiency of the target cells (e.g., HSC or T cells). To have a robust method, it is important to have good reliable cell line stock, and a positive control. To have a reliable test, we need to control all these parameters, starting from the beginning of the development of the process, to ensure the same robust method is used throughout.

**EJ:** From our perspective, we use analytics primarily when developing new cell lines and reagents to monitor and validate the changes and improvements we make. We look at typical things such as particle titer, genome titer, and infectious titer, with challenges including variability of assays.

**RL:** We use conventional methods, such as PCR and ELISA by default. There are new tools now, such as equipment based on microfluidics or optics, which are beginning to be used more frequently, together with conventional methods. Automation of both new and traditional assays is helping us to use a smaller sample volume and get quicker analytics.

**Q** What is your opinion on full versus empty analysis in LV vector manufacture?

**SM:** It has not been a priority as it has been in AAV, primarily because, up to now, LV has been mostly *ex vivo*. Considerations about the load of empty particles into a patient are not as high, but there are tools out there, including high-performance liquid chromatography (HPLC)-based methods, being used for this. As we do start to move more into *in vivo* therapies for LV, this will become an important CQA.

**Q** For analytical requirements, is total particle count important?

**SM:** It is certainly an attribute that we measure using P24 ELISA or other methods. Even though we are mostly focused on infectious titers, understanding the particle-to--activity ratio goes back to the question of empty versus full.

**Q** What are the considerations and best practices to ensure robustness relating to assay selection and evaluation?

**MN:** When we select an assay, even if it is based on a commercial kit, for example the P24, we need to exercise qualification to reduce further variability of the analytical methods. It is important to take into consideration interference studies in the process, as in each step, the vector is in a different media or buffer. These buffers could affect the results, so we must evaluate this interference to ensure that the analytical methods are fully reliable and reproducible.

**SJ:** The analytics with LV must be robust, quantifiable, qualifiable, and eventually validatable in commercial productions. Knowing your assays, how they work, and what the pitfalls are is paramount.

**SM:** The big question is, 'do we have the assay under control'? We want low variance and high repeatability. One of the most important attributes of the assay is the ability to have different people do it – on different days, using different instruments – and still get the same answer. When assays are performed incorrectly, we should be investigating the impact of those changes on the assay itself to build a better understanding of how robust the methods are. If we notice that small changes are dramatically impacting the assays, it hints that the assay is not robust. We need to start building a library of investigations, to determine what are the critical parts of an assay and how they can be negatively impacted.

**RL:** In terms of analytics and how they contribute to process development, infectivity and viral titer is the key parameter. It is key to control the limits of the assay, and then identify the factors that affect the assay.

**Q** What will be the key next steps for bioprocess and analytical innovation in the LV field to drive further scalability and quality/consistency improvements?

**SM:** The need for rapid in-process analytics for viral vectors still remains. This is a particular concern due to the fragility of the LV particles themselves. We want to minimize hold and processing times as much as possible and to do so we need rapid, reliable analytics. Focusing on rapid particle titering or GOI titering is going to be a key driver for scalability, product quality, and consistency.

The introduction of commercially available 'off-the-shelf' solutions is also going to be an important part of developing consistent manufacturing processes, helping to standardize the analytics across the industry. Right now, the use of different methods makes it difficult for us to make comparisons and causes challenges for regulators in setting industry guidance.

**RL:** There are three key components for bioprocessing. One is the development of more producer cell lines to increase productivity. Second, automation is key, both in process and analytics. The third key point is the development of new serotyping strategies that better target the cell to be transfused, and the development of transduction enhancers. This is key to developing more cost-effective processes so that more patients can be treated.

**MN:** Another step, from a process point of view, is the reduction of the dead volume in downstream instruments. The systems that are now on the market for concentration have been created for large-scale processes and the dead volume in the instruments is limiting the vector concentration. Suppliers should help us with having more flexibility in size and measure for the single-use instruments.

**EJ:** One key aspect is ensuring early setup with products and tools that have a clear path to commercial launch. Historically, a challenge in this space has been the lack of fit-for-purpose reagents. Starting early in development with serum or animal-containing components makes things more complicated from a downstream processing or regulatory point

of view. Therefore, the components in a process that are intended to be used in cell and gene therapy applications must be identified and come with appropriate product documentation to maintain a streamlined path to the clinic.

## Q Which parameters do you need to optimize for scaling up your process?

**EJ:** Optimizing the density the cell line is grown at and transfected at are both key. In our experience, there are many aspects to optimize at the transfection step, such as plasmid DNA, ratios of plasmids, the timing of complexations depending on transfection agent, and harvest time. We see various impacts from optimizing each of those steps, including benefits in terms of the titer.

## Q How might the evolving regulatory landscape impact the picture?

**RL:** In my experience as QP, as viral production has developed and knowledge has increased, the number of applications for gene therapy has also increased. Regulatory bodies are increasing their demands for process control and products of a higher quality. This directly impacts the standards for LV manufacture and prioritization. We need to control more CQAs to tighter certifications. We are looking for products of better quality, with fewer contaminants.

**SM:** Regulators want us to demonstrate our processes are controlled, both in the consistency of processing and in product safety and quality. We must consider and monitor the residuals that end up in the product due to the process that we have. Regulators require safety first and foremost, which should be our major focus.

**SJ:** The landscape has changed tremendously over the last 5 years. Regulators have placed great importance on quality and ensuring that we are monitoring our processes. Even from the early stages of process development, it is recommended to think about the final stages of commercialization. Understanding your process, with quality in mind from the beginning, is important to ensure you can get through the regulatory pathways. This ensures patients are safe, which is of primary importance.

## Q What are the key challenges to overcome to enable greater automation – for example, in leveraging in-process analytics?

**MN:** There has been good innovation within in-line process controls, including the recent Raman spectroscopy technologies. This kind of technology, despite its promise, is very demanding in terms of resources to be invested. It needs a dedicated team to interpret the data and evaluate the metabolites that best correlate with the CQAs. We are now

scouting and evaluating these new technologies because they offer an interesting opportunity to expedite process development.

## Q What is your experience in trying to implement greater automation levels in the manufacturing process?

**SJ:** To make the best possible analytical technology from beginning to end, thinking about the future is important. Understanding the process and having a way to look at every parameter, from the bioreactor to the chromatography, is ideal. We want to move towards full automation of processes.

**SM:** One of the largest barriers to entry is cost. Instruments are expensive and one key challenge we have right now is being able to deploy these instruments. The second thing is matrix interference as a key barrier to ensuring that we have reliable process analytics, especially in upstream bioprocessing. This can be overcome, but there is potential for miscalibration or poor data due to matrix issues.

**RL:** Automation is expensive, but if it is correctly implemented, it is cost-effective. It should be seen as an investment decision by the company. It must be qualifiable, validated, and on time.

## Q In downstream processing of LV vectors, is tangential flow filtration (TFF) done before chromatography, and what kind of chromatography do you use to purify and concentrate your vector?

**MN:** Regarding chromatography for LV vectors, we now use anion exchange chromatography. To ensure GMP, we use ready-to-use columns. Regarding TFF, our approach has been to develop the adherent process with a hollow fiber step after chromatography as an additional vector concentration step. For the suspension system, we are evaluating the addition of a first fiber concentration step at the beginning of the downstream in order to reduce the volume of the bulk vector prior to proceeding with purification. This step could be essential when the USP scale will increase to 1000-2000 L or more.

## Q When it comes to chromatography, which specifications are important, and where do you see gaps in the currently used media?

**MN:** For the development of the chromatography step, we consider the quality of the vector that we obtain. The suspension after clarification is still rich in proteins and host cell DNA, so there is a need for a stronger endonuclease step. We are working on the chromatography, fine-tuning the amount of resin needed for the vector. At the end of the chromatography step, we are looking at yield, in terms of physical particles, and the infectivity

and stability of the vector. Moreover, we evaluate the presence of impurities, mainly host cell proteins, total DNA, and other product and process-related residuals.

## Q How do innovations in LV vector processing and analytical toolkits impact decisions on whether to outsource LV process development and manufacture or keep them in-house?

**SJ:** From a small biotech perspective, we need CDMOs to produce these viral vectors. A virtual company that may have an office space in a large lab may not even have the facilities to do process development. They need to rely on partners and collaboration between teams to get this work done. At CDMOs, there are subject matter experts (SMEs), who spend time ensuring that we are successful.

**EJ:** We are always going to see the need for CDMOs and CMOs. Greater standardization in analytical assays and bioprocessing solutions is going to make transfer easier, whether you start early development in-house and then outsource, or vice versa.

**RL:** LV manufacturing is such a complex service, and needs such a degree of optimization, that outsourcing can be much more reasonable. From an economic point of view, the costs associated with a GMP facility with trained personnel are so high that outsourcing is often a good idea.

**MN:** The cost and the setting of the quality systems for GMP manufacturing are so well-established in CDMOs, that small-to-medium companies need to rely on that experience for vector manufacture, particularly in a clinical setting.

**SM:** CDMOs are key to ensuring that we get these drugs to patients as soon as possible. The complexity and the amount of investment in infrastructure and equipment are very high that it is a huge barrier to execution. Turning to the analytical side, as more kits are commercially available, it changes the dynamic, making it easier for people to in-source some of these assays that previously had to be outsourced. Even with that, there is certainly going to be space for CDMOs to help deliver those products to patients.

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## AUTHORSHIP & CONFLICT OF INTEREST

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INNOVATOR INSIGHT

# Simplifying lentiviral downstream processing with a novel affinity resin & robust analytical tools

Chantelle Gaskin & Suzy Brown

Recombinant lentivirus has become a vector of choice for many gene-modified cell therapies, including several US Food and Drug Administration-approved cell therapies, due to its broad tropism and long-term, stable gene expression in non-dividing cells. The safety and efficacy of lentiviral-based therapies depend greatly on optimized and controlled lentiviral production. Downstream purification of lentiviral particles presents unique challenges, and robust analytics are critical to verify both the recovery and infectivity of the purified product. In this article, an overview of challenges and newly developed solutions for robust lentiviral purification and rapid analytical methods for titer determination and impurity quantification will be presented. Details of a new affinity chromatography resin to purify vesicular stomatitis virus glycoprotein pseudotyped lentivirus, as well as two qPCR-based genomic and proviral infectious titer assays, will be discussed.

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As of the first half of 2021, there had been an estimated 288 cell and gene therapy programs in clinical trials using lentiviral vectors. Of US Food and Drug Administration (FDA)-approved cell and gene therapies, there are four CAR-T cell therapies and one *ex vivo* gene therapy that use lentiviral vectors. Characteristics of the recombinant lentiviral system, including long-term transgene expression, high packaging capacity, and the ability to transduce both actively dividing and non-dividing cells are advances used in *ex vivo* gene-modified cell therapies. Lentiviral vectors have also found use in therapeutic gene editing and genetic vaccine platforms. As a result, the demand for high-quality lentiviral

vectors for therapeutic applications continues to be strong.

To meet the growing demand for lentiviral vectors, advances have been made to improve yield and turnaround in large-scale production, with robust analytics to ensure vector quality and safety. From a manufacturing perspective, lentivirus is produced using cell-based bioproduction systems of adherent or suspension HEK293, or 293T cell lines, co-transfected with multiple plasmids carrying transgene packaging and enveloped elements to assemble into the recombinant viruses. Stable producer cell lines can also be used. Viral vectors are then purified, concentrated, and formulated. Although the manufacturing process is similar to other viral vectors, the characteristics of lentiviral vectors are different, which needs to be accounted for in downstream purification and analytics.

## PROCESS-RELATED SOLUTIONS FOR LENTIVIRAL PRODUCTION

Thermo Fisher Scientific offers a complete solution for the production and purification of lentiviral vectors. The Invitrogen GeneArt™ brand services provide flexible, reliable custom gene synthesis, with short turnaround times. For vector production, the Lipofectamine™ 3000 transfection reagent for adherent cell systems produces efficient transfection and high titers with lower reagent requirements. The LV-MAX™ system is a next-gen lentivirus production system and can be used alongside the newly launched CaptureSelect™ lentivirus affinity resin for vector purification.

The LV-MAX™ lentivirus production system includes high-density HEK293 suspension cells, chemically defined media, production supplements, transfection reagent, optimized lentiviral packaging plasmid, and a novel enhancer reagent. The system was designed to be scalable with no animal-derived components and includes regulatory support files for GMP manufacturing. Customers have been able to increase their titers 10-fold using the LV-MAX system.

## *MYCOPLASMA* TESTING

At lentivirus vector harvest, manufacturers need to test for *Mycoplasma* to ensure product quality and patient safety. While there are several available *Mycoplasma* testing solutions, few meet the sensitivity, specificity, and robustness required for regulatory agencies. One available solution that meets these criteria is the MycoSEQ™ assay.

The MycoSEQ™ Mycoplasma detection system has been designed to fulfill the regulatory guidance in European Pharmacopeia section 2.6.7 on *Mycoplasma* testing with nucleic acid-based methods. The qPCR-based system provides clear, objective, multiparameter data interpretation using three acceptance criteria for the identification of a positive result. The assay is proven to detect over 90 different *Mycoplasma* species, with no cross-reactivity to off-target bacterial organisms. It is also highly sensitive and enables validation of less than/equal to ten genome copies per mL in test samples. This off-the-shelf kit also contains a patented discriminatory positive control that can eliminate the concern of any potential cross-contamination, as it is easily differentiated from a true positive result.

A global support network of experienced Field Application Scientists (FAS) can help deliver full workflow training, from sample preparation to results interpretation. Additionally, a drug master file is held with the FDA for this solution, and Thermo Fisher Scientific offers in-house experience in validation design and regulatory support. Instrument installation qualification/operational qualification (IQ/OQ) services and computer system validations are provided for the integrated data analysis AccuSEQ™ software, which features 21 CFR part 11 compliance features.

Following validation, regulatory filing, and review, customers have received regulatory acceptance to use the MycoSEQ assay for lot release testing applications across multiple therapeutic modalities. This includes cell culture manufacture, cell therapy, and tissue therapy.

Over 40 customers have already received regulatory approval for a drug filing that uses the MycoSEQ assay for *Mycoplasma* testing.

## PURIFICATION CHALLENGES

The most cited purification challenges in the field stem from the fact that lentivirus is an enveloped virus. It requires processing within narrow ranges of pH, temperature, conductivity, and shear. Many current purification processes either have low feasibility for scale-up or require longer processing times to produce the required levels of purification, such as centrifugation and tangential flow filtration. Longer processing times also translate to lower recovery. There are also several anion exchange processes that use different chromatographic support technologies – monolith or membrane absorbers – to reduce process time. However, anion exchange typically requires exposure to high salt concentrations, which might impact the infectious titer. Current purification methods are also unable to distinguish between infectious product and product-related contaminants.

One of the most requested solutions has been an affinity chromatography method that could leverage gentle buffer conditions and reduce processing times while retaining infectious titers. In general, current methods do not typically yield more than 30% recovery. In many cases, process development scientists are seeing even lower recoveries than that.

To understand why we see low recovery, we must look at the lentiviral particle structure. The genetic payload is encapsidated by capsid proteins such as P24 and enveloped proteins such as vesicular stomatitis virus glycoprotein (VSV-G). Lentiviral tropism is determined by the ability of the enveloped proteins to interact with the receptors on the cell surface. VSV-G is one of the most used pseudotypes in cell therapy due to the broad tropism across different species and cell types.

During production in human cell lines like HEK293, a variety of particle species are generated in addition to the infectious viral particles. These are product-related contaminants: virus-like particles (VLPs), non-infectious particles, and exosomes, with and without envelope proteins. The envelope proteins found on the infectious particle are fragile and sensitive to the conditions that are commonly used in the purification of hardier molecules like monoclonal antibodies (MABs). This leads to low recovery during processing.

## ANALYTICAL CHALLENGES

Analytical methods for lentiviral quantitation include the P24 ELISA for capsid-based titers, reverse transcriptase (RT)-qPCR or digital PCR for genome-based titers, and particle counting systems for all physical particles in a sample. A combination of these methods is often used. Each method has its own advantages and disadvantages but regardless of the method used, manufacturers face challenges including poor reproducibility, high variation, difficulty optimizing assays in complex matrix conditions, and inefficient recoveries. These challenges, coupled with the lack of a lentivirus reference standard, make it difficult to accurately quantify yields.

Once cells are transduced with lentiviral vectors, they must be tested to ensure the safety, quality, and potency of the gene-modified cell therapy product. There are several different ways to assess integration and lentiviral infectivity, including flow cytometry or fluorescent-activated cell sorting, which look at transgene expression, whereas qPCR and digital PCR measure copy number of the provirus in the genome itself. Cell-based assays can be used to calculate the infectious titers of the lentiviral vectors. As high integration may be a safety risk, these assays must be sensitive and accurate. Vector copy number (VCN) is tested for each lot of a transduced cell product, so the assay must be amenable to use in a quality control (QC) environment with rapid turnaround and minimum manual intervention preferable.

Cell lines and healthy donor cells used for infectious titers may have different transduction efficiencies to patient cells, which poses additional challenges. Frequently, transgene-specific assays have been used, which measure multiple attributes of a final product. However, this limits the ability of an assay to be used across a program as a platform approach and will add to assay development and program timelines.

## SOLUTIONS FOR LENTIVIRAL PURIFICATION & ANALYTICAL TESTING

The newly launched CaptureSelect™ Lenti VSV-G Affinity resin was designed for specificity to VSV-G pseudotyped lentiviral vectors. The resin provides high-level purification in a single step with gentle elution conditions at neutral pH to maximize infectious particle recovery.

To determine the dynamic binding capacity, experiments were performed using a 1 mL, 3 cm bed-height column, equilibrated in 50 mM HEPES, 150 mM salt pH 7.5 (Figure 1). The titers of the load material were determined by p24 ELISA to be $4\times10^9$ particles per mL. Flowthrough fractions were collected in 5 mL increments and sampled for titer determination. As can be seen from the breakthrough curve, there was a 10% breakthrough corresponding to $1\times10^{11}$ particles per mL total capacity.

On a 10 ml CaptureSelect Lenti VSV-G column (1.6×5 cm) equilibrated in 50 mM HEPES, 150 mM NaCl pH 7.5, 250 ml clarified suspension harvest with a titer of $1.1\times10^{10}$ total particles/ml was loaded at a flow rate of 5 ml/min (150 cm/h, 2 min residence time). The load was washed out with equilibration buffer and the column was eluted with 50 mM HEPES, 150 mM NaCl, 0.8 M Arginine pH 7.5 (Figure 2).

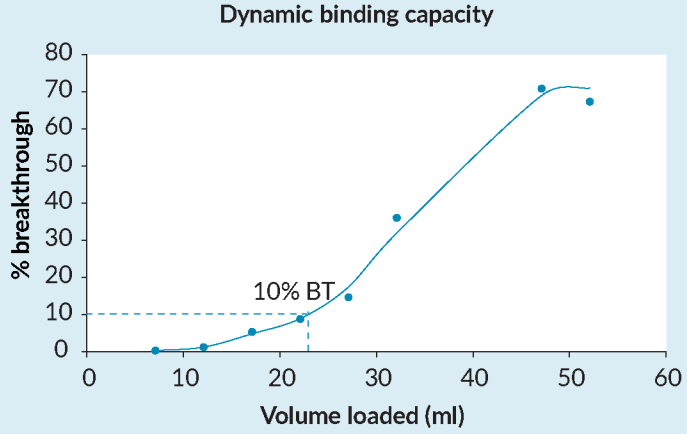
The elution was efficient and showed good compatibility with the enveloped virus particles, resulting in high concentrations of infectious particles in the elution fraction. Depending on the feed and application, optimization of the elution buffer might be needed with adjustments of the arginine concentration, pH, or combinations thereof.

A summary of the recovery results by total and infectious particles, as determined by p24 ELISA and a cell-based infectivity assay, is shown in Table 1.

This data demonstrates that the infectious particle titer increases after purification. As the process progresses, the total to infectious particle ratio decreases. This results in a more

## FIGURE 1

**Dynamic binding capacity determination on a 1 mL column.**



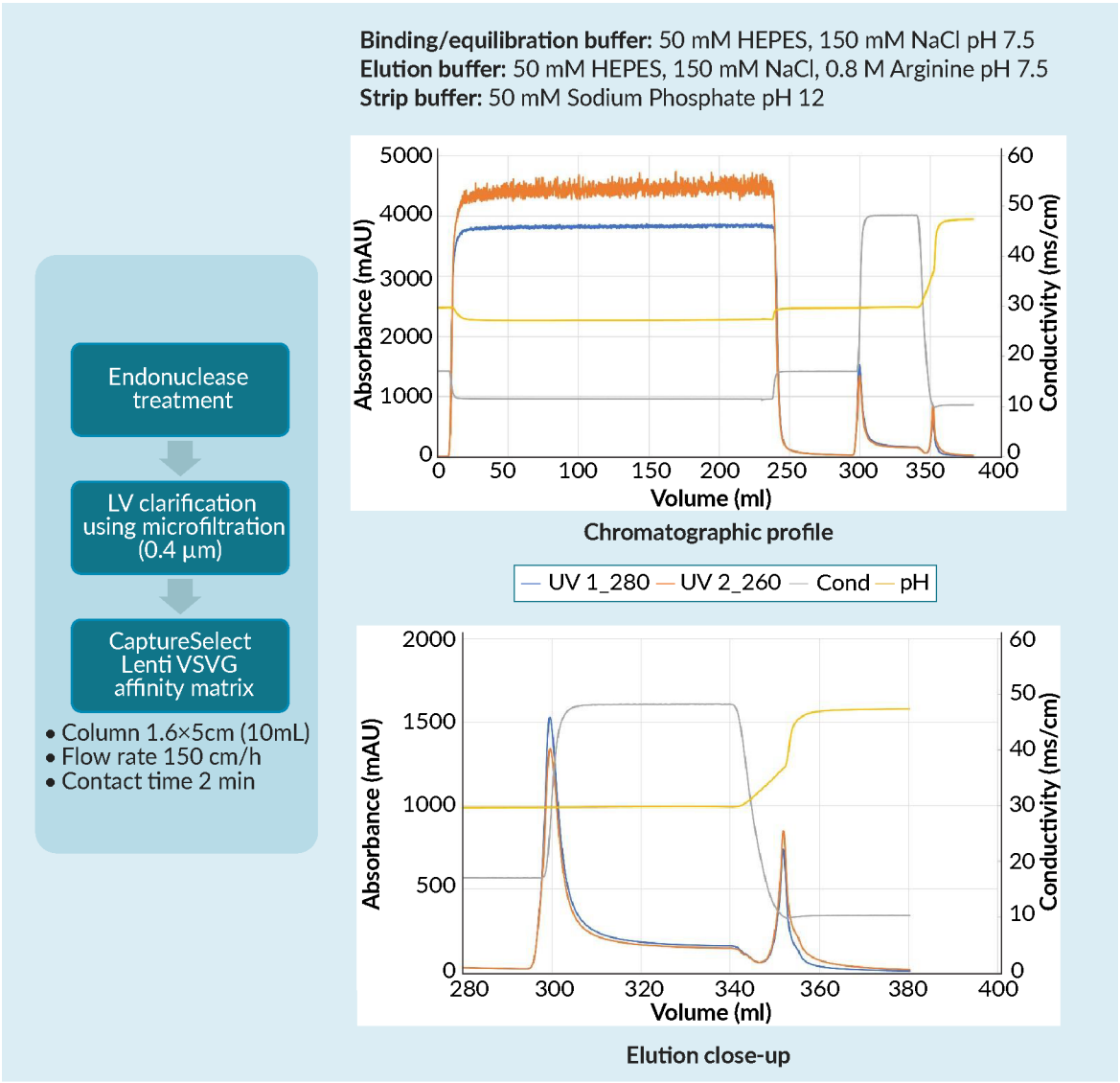

DBC is determined by P24 total particle ELISA

| Fraction | Volume (mL) | TP/mL | $C/C_0$ | |
|---|---|---|---|---|
| Start | | 3.98×9 | | |
| 1 | 7.1 | 1.55×7 | 0.39 | |
| 2 | 12.1 | 5.24×7 | 1.32 | |
| 3 | 17.1 | 2.14×8 | 5.39 | |
| 4 | 22.1 | 3.53×8 | 8.88 | 24.6 mL |
| 5 | 27.1 | 5.85×8 | 14.7 | |
| 6 | 32.1 | 1.44×9 | 36.09 | |
| 7 | 47.1 | 2.82×9 | 70.84 | |
| 8 | 52.1 | 2.68×9 | 67.33 | |

- 10% breakthrough ($C/C_0$ =10%) estimated from the curve at 24.6 ml loading
- This relates to 9.78×10 total particles/ml resin (= 1×11)

## FIGURE 2

**Representative chromatogram using recommended process conditions.**




than 5-fold enrichment of infectious particles with a 50–60% recovery in the column eluate.

An enrichment of infectious particles is expected with the Lenti-VSVG resin due to selectively binding the VSV-G envelope protein on the capsid. The P24 and other capsid proteins will be present in non-infectious particles, whereas the VSV-G protein is present in a subpopulation of particles including the infectious particles. An 85% DNA clearance and over 95% protein clearance were also achieved.

The Lenti VSV-G ligand is immobilized onto a 65 µm highly crosslinked agarose bead. The resin has a pressure rating up to two bar with a recommended velocity of up to 200 cm per h. It is shipped in 20% ethanol and in 5-, 10-, and 50-mL bottle sizes.

## ANALYTICAL TOOLS FOR PURIFICATION PROCESS DEVELOPMENT

As lentiviral vectors are used to transduce cells, they are an active ingredient in drug

substances. Drug substances must be tested for critical quality attributes (CQAs) in-process and at lot release according to FDA chemistry, manufacturing, and control (CMC) guidance. Lentiviral vector CQAs include identity, purity, strength, safety, and quality. Titer or vector concentration is an important attribute for strength, quality, and safety testing.

For lentiviral characterization and integration analysis, Thermo Fisher Scientific has recently introduced two new qPCR assays. The ViralSEQ™ Lentiviral Physical Titer Kit is a one-step real-time RT-qPCR assay for genome-based lentiviral titers, measuring physical titer in viral particles per mL. Additionally, the ViralSEQ™ Lentivirus Proviral DNA Titer Kit is a qPCR assay to measure integrated lentivirus or proviral copies in transduced cells. It can be used to calculate infectious viral titers and VCN. Combined, these two assays provide a convenient method to compare qPCR to qPCR data, for total and infectious titers, as well as for measuring VCN for analytics across the lentiviral workflow. Both assays are designed to provide robust performance and facilitate lentiviral analytics, in-process development, and manufacturing in QC environments.

The assays are just one component of the experimental workflow. Thermo Fisher Scientific provides an integrated solution to meet your needs for a complete workflow, from sample preparation to data analysis. Both lentiviral assays have been optimized for this workflow, enabling manual or automated sample preparation on the KingFisher™ Flex platform, using PrepSEQ™ nucleic acid sample preparation kit. The RT-PCR will be run on a QuantStudio™ 5 instrument, and data analyzed using the AccuSEQ software, which enables 21 CFR part 11 compliance in GMP environments. Thermo Fisher Scientific can also support other applied biosystems or qPCR instruments through instrumentation and validation.

## Lentivirus physical titer kit

The ViralSEQ Lentiviral Physical Titer Kit is an RT-qPCR assay for the quantitation of genome-containing lentivirus vectors. The assay targets a conserved long terminal repeat (LTR) region in the lentiviral genome. As this region is critical to integration into cells, most lentiviral production systems have conserved LTR regions. This assay can be used across production lots and programs if they all use the same vector system. The TaqMan™ chemistry used in this assay provides high target specificity, preventing background signals from potential cross-contaminants, such as residual plasmid or host-cell DNA, from overestimating titers. The assay has over seven logs of dynamic range, from 50–$10^9$ copies. This actively quantifies a whole range of lentiviral yields. The kit also includes all the reagents required for the RT-qPCR reaction and comes with an RNA standard.

The physical titer kit total assay runtime, including the sample preparation, is under 6 h. The individual steps include the preparation of reagents and sample dilutions, nucleic acid extraction, a DNase treatment to remove any residual DNA, PCR reaction prep, and RT-qPCR run with data review.

TABLE 1

**Comparison of total particle to infectious particle ratios.**

| Sample | TP/mL | IP/mL | TP/IP ratio | Recovery | HCP removal | Total DNA removal |
|---|---|---|---|---|---|---|
| 1. Feed | $1.10\times10^{10}$ | $7.98\times10^{7}$ | 138 | | | |
| 1. Flow through | $3.25\times10^{8}$ | $8.30\times10^{5}$ | 392 | | | |
| 1. Elution | $4.44\times10^{10}$ | $4.42\times10^{8}$ | 100 | 50% | 99% | 80% |
| 2. Feed | $1.11\times10^{10}$ | $9.00\times10^{7}$ | 165 | | | |
| 2. Flow through | $1.28\times10^{9}$ | $5.45\times10^{6}$ | 245 | | | |
| 2. Elution | $2.6\times10^{10}$ | $4.66\times10^{8}$ | 71 | 58% | 97% | 97% |

## FIGURE 3

**ViralSEQ Lentivirus Physical Titer Kit standard curve and amplification plots.**

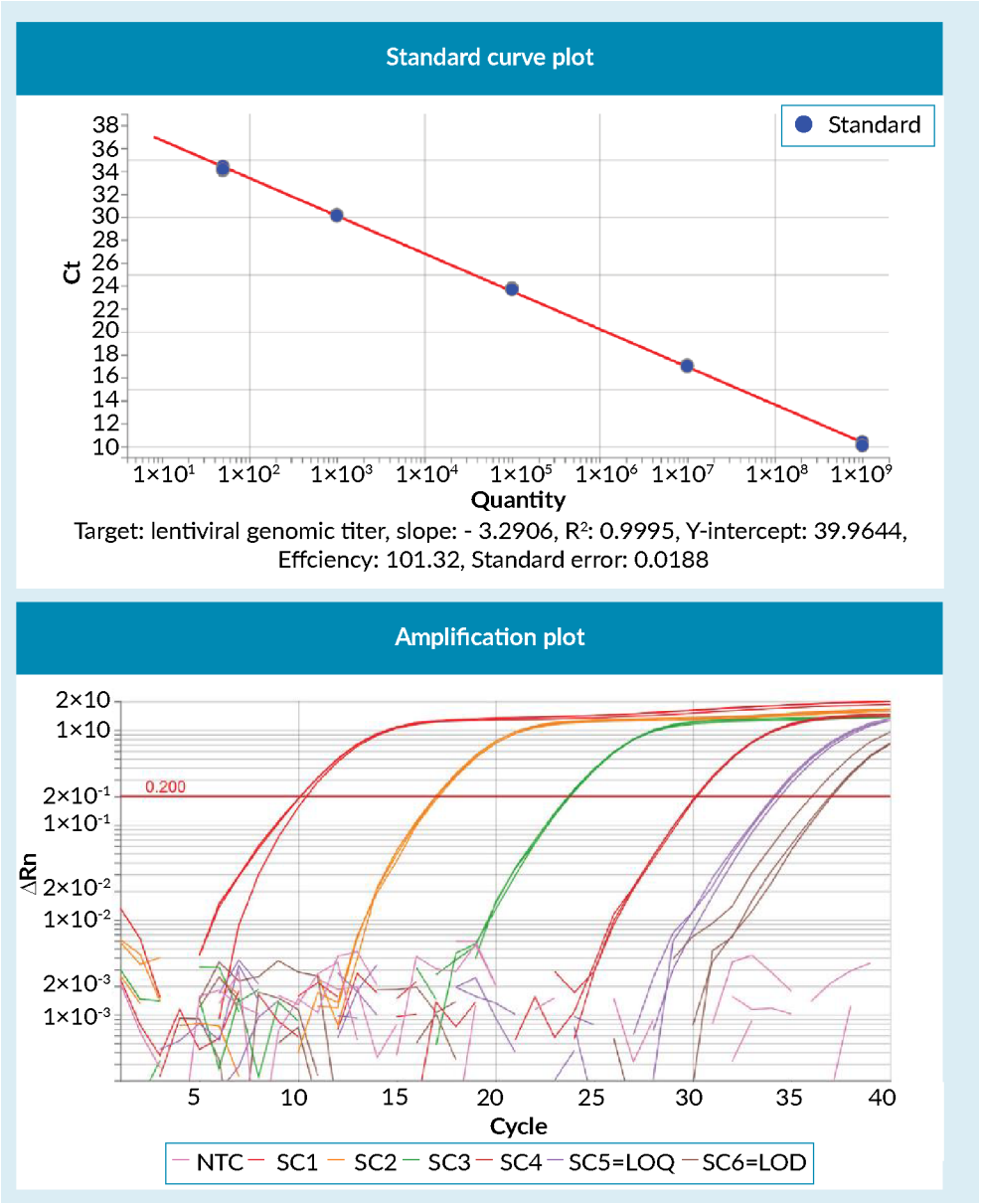


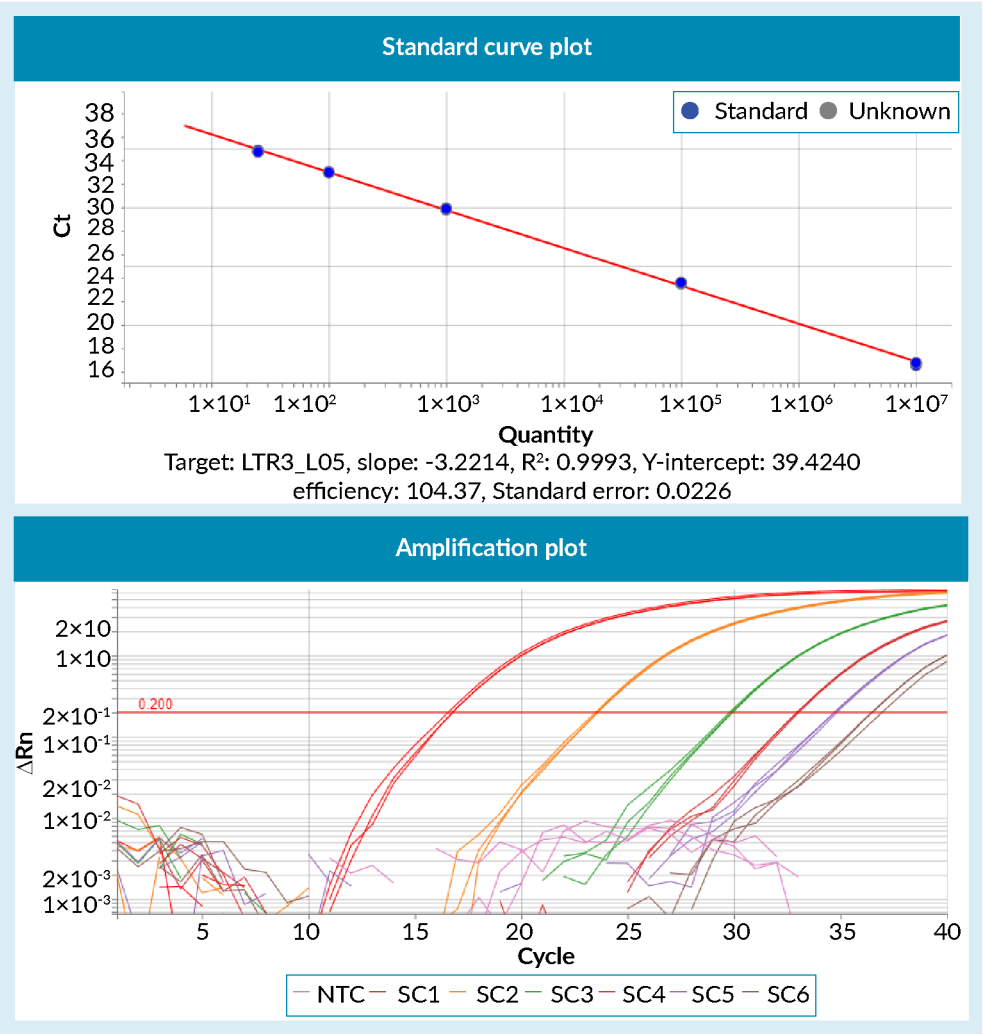

Figure 3 shows a representative standard curve plot with a PCR efficiency of 101% and an $R^2$ of 0.999. The amplification plot shows the amplification curve for the standard curve point across the assay range, from 50–$10^9$ copies per reaction.

## Lentivirus proviral DNA titer kit

The ViralSEQ Lentivirus Proviral DNA Titer Kit is a qPCR assay that also targets the LTR region in the integrated vector copies for transduced cells, making it suitable across lentiviral programs that use the same system. Data from this assay for proviral copy numbers can be used to calculate the lentivirus infectious titers and the VCN for transduced cells. The TaqMan™ chemistry provides high target specificity, and the assay range enables proviral copy number for a range of transduction efficiencies. The assay has excellent sensitivity with a limit of quantification of 25

## FIGURE 4

**ViralSEQ Lentivirus Proviral DNA Titer kit standard curve and amplification plots.**




copies per reaction. This kit comes with all reagents required for the qPCR and a DNA standard control.

The proviral DNA titer assay takes ~5 h including sample preparation. The workflow includes the preparation of reagents and serial dilutions, sample extraction, qPCR preparation and run, and final data analysis. The representative standard curve plot in Figure 4 shows a PCR efficiency of 103% and an $R^2$ of 0.999. The amplification curves for the standard curve points across the assay range from 25–$10^7$ copies per reaction.

## ASSAY DEVELOPMENT & VALIDATION TESTING

There are many regulatory expectations regarding the characterization of lentiviral vectors, transduced cells, and the validation of analytical methods that are used for quality testing of this type of product. There is a need for validated assays in each specific process that manufacturers perform as part of their CMC filing. Therefore, Thermo Fisher Scientific offers assays with verification and internal validation testing to ensure that these assays perform to the high standards required to meet validation criteria and regulatory expectations.

As part of development testing, bioproduction and cell culture matrices have been evaluated to mimic the representative sample conditions customers will experience and qPCR data is correlated against orthogonal titer methods. For internal assay validation, multiple assay lots have been tested with multiple operators across multiple sites, to ensure robust, reproducible assay performance. Both manual and automated sample extraction have been compared, and two different qPCR systems have been used.

## Internal validation for the proviral DNA titer kit

To measure site reproducibility, data was generated by different operators run on different instruments for Site 1 and Site 2. As shown in Figure 5, both sites performed similarly for standard curve metrics for PCR efficiency, $R^2$, Y-intercept, and slope. The assay shows good site-to-site reproductivity for standard curve performance.

Assay precision was evaluated across multiple variables, with DNA controls from three different kit lots at standard curve concentrations. The percentage coefficient of variability (CV) criteria (less than/equal to 30%) was achieved for all data points.

Assay specificity was evaluated by testing for any cross-reactivity using a panel of DNA from potential cross-reactants, such as process-related impurities including HEK293, *E. coli*, baculovirus, as well as plasmids containing ampicillin or kanamycin resistance genes. The assays were tested in two separate runs, and no cross-reactivity was detected for any of the species tested.

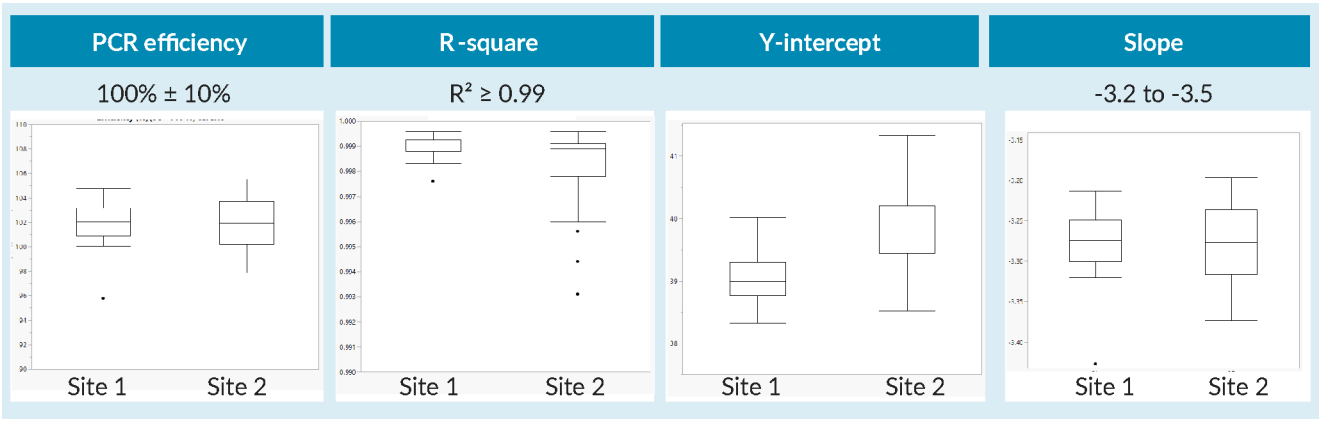
To evaluate sample preparation recoveries, the DNA control from the kit was spiked into a test sample matrix at 500 and $2.5\times10^6$ copies. To mimic cell culture conditions, a test matrix of 50% cell culture medium with HEK293 cell lysate at $10^5$ cells was used. Extraction was then performed using the PrepSEQ™ nucleic acid extraction kit on the KingFisher™ Flex automated platform, as well as manually. The criterion for recovery was set to 70–130% and the data obtained from the KingFisher Flex platform and the manual extraction were all within this range.

To test the performance of the proviral DNA assay with a representative transduced cell sample, two cell lines that have been transduced with lentiviral vectors were sourced from our collaborators. The qPCR copy numbers were determined with the titer kit and a corresponding VCN was calculated. The data points were compared to an orthogonal test method, and the two methods showed a good correlation.

In summary, the ViralSEQ lentiviral titer kits provide a rapid, robust, and reliable

FIGURE 5

**Proviral DNA titer kit: PCR performance.**



solution for measuring genomic and proviral copy numbers for lentiviral characterization. Both assays quantitate based on the LTR region and facilitate analytics across the workflow from lentiviral vectors to integrated proviruses. The assays have been internally validated to support customer validation at user sites as per regulatory expectations.

# Q&A


**David McCall,** Editor, BioInsights speaks to (pictured left to right) **Chantelle Gaskin,** Field Application Scientist, Viral Vector Purification and Downstream Process Development, Thermo Fisher Scientific and **Suzy Brown,** Senior Field Application Specialist, Pharma Analytics, Thermo Fisher Scientific

**Q** Can the affinity resin be used for other pseudotypes other than VSV-G?

**CG:** We have not generated any data yet, but the ligand was developed for specificity against the VSV-G enveloped protein.

**Q** In my opinion, a 25 mL max load for a 1 mL column sounds low. Is there a way to increase the capacity?

**CG:** Lentivirus is around 100 nm in diameter, so if you consider the difference in size between a lentiviral vector versus an AAV vector or a smaller molecule like a monoclonal antibody, you can expect the accessibility of that molecule to the surface area of the resin is going to be lower.

Typically, to increase capacity, we recommend increasing residence time or optimizing your pre-chromatography unit operations. Customers might want to start with a two-minute residence time initially, considering a balance between maximizing recovery and maximizing process productivity.

**Q** How do I know these lentiviral titer assays will work for our recombinant lentiviral platform?

**SB:** The two ViralSEQ Lentivirus kits have been designed against one of the conserved regions of the LTR sequence of the plasmid delivering your transgene.

We have tested that sequence *in silico* and found that it is compatible with over 200 lentiviral transfer plasmids available, including a few that have self-inactivating modifications. If you are using a conserved LTR, our assay will work with most transfer plasmids.

If you have made specific modifications in the LTR sequence, then the best way for us to address this is to contact us directly so we can check the compatibility of your sequence with our primers.

## Q What is the base bead of the lentiviral affinity resin? Is there a POROS™ bead backbone available?

**CG:** This resin has been years in the making. The newly launched resin is based on highly cross-linked agarose, but the team did initially develop different resin prototypes on both the agarose and POROS base matrix. The crosslinked agarose matrix had a better performance profile, so that was chosen to move forward.

## Q Do you have any prepacked columns with the lentiviral resins?

**CG:** Right now, we only have bulk resin formats available: 5-, 10-, and 50-mL bottle sizes. We are currently working on prepacked columns and are trying to get customer feedback to see what column formats or column dimensions would be most useful.

## Q Can the physical titer assay distinguish between plasmid and viral genome?

**SB:** The physical titer assay does not distinguish between plasmid or viral genomes. However, to mitigate any residual plasmid in your sample, which could lead to titer overestimation, we have included a DNA removal step as part of the workflow for that kit. You will first extract total nucleic acid, then perform your DNase treatment, which will remove any potential residual plasmid DNA and any host-cell DNA that is carried over. Following reverse transcription, during the qPCR, your primers and probes will then specifically bind to the cDNA and amplify the target LTR sequence there.

We are not able to directly distinguish between plasmid and viral genome, but we have taken steps to address any potential residual plasmid during the workflow.

## Q Can your physical titer assay be correlated with transduction assays or bioassays?

**SB:** In terms of the correlation between total virus particles versus infectious titer, the two assays that we have discussed can be used in parallel, and then the

data can be compared. This means you are correlating results between qPCR methods. It is much easier to correlate two qPCR methods than one qPCR method and another, such as flow cytometry.

The correlation that we typically see is a 2–3 log difference between the two titers. We have heard from customers that the expectation is that for every one particle that may be infectious, there may be 100 that are not infectious. This will depend on the quality of your lentiviral vectors and the purification process being used.

## Q How many times can the lentiviral resin be re-used?

**CG:** This is a newly launched resin, so comprehensive applications data packages are not yet available. We do have a few internal studies ongoing to be able to answer that and other questions.

We know that an effective cleaning strategy is critical to the reusability of the resin. So far, phosphate at pH 12 is our recommended strip buffer based on our data.

## Q Will the lentivirus qPCR assays work on digital PCR platforms?

**SB:** The two lentivirus titer kits have been developed and optimized for a qPCR system only. Although we now offer a digital PCR instrument, the QuantStudio™ Absolute Q™ digital PCR system, and we have done some initial feasibility work on this, we cannot specify the performance criteria for digital PCR at this time.

## Q Can I use other qPCR instruments for the assays than those presented in the validation study?

**SB:** Yes – all the analytical kits either use TaqMan or SYBR™ Green chemistry and can be run on any qPCR system. If you have the correct channels to detect the fluorescent dye, or label being used, you can use them.

The reason we talk about the QuantStudio 5 and the 7500 Fast instruments is that we have shown they are validatable and can be utilized with our AccuSEQ software. This software, as well as enabling the 21 CFR part 11 compliance, has been designed to support these specific assays with in-built templates, automated calculations, and presence-absence calls.

## BIOGRAPHIES

**CHANTELLE GASKIN** is a Field Applications Scientist, specializing in protein and viral vector purification and downstream process development. She held leadership positions at Applied Genetic Technology Corporation and Brammer Bio, prior to joining the Thermo Fisher Scientific Bioproduction Division in 2020. With over 10 years of experience in gene therapy,

Chantelle has accumulated comprehensive knowledge of standard industry practices and regulatory standards, applying this knowledge to advance development of therapies for a variety of indications including ocular, CNS and systemic disease. Chantelle holds a master's degree in Chemistry from University of Florida and a Bachelor's in Chemistry from Smith College.

**SUZY BROWN** is the Senior Field Application Specialist for Pharma Analytics, supporting customers in the UK and Ireland. She has been with Thermo Fisher Scientific for over 5 years where she provides training and implementation of solutions for contaminant and impurity testing designed for the cGMP environment. Prior, she worked in the biopharmaceutical industry as an Analytical Development Scientist for 3 years at Allergan Biologics, within a Molecular Biology team and has experience in molecular methods and impurity testing. Suzy holds a PhD in Developmental Biology as well as a Bachelor's in Cell Biology from University of Manchester (UK).

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## PRODUCT USE STATEMENT

**ViralSEQ:** For research use only. Not for use in diagnostic procedures.

## AUTHORSHIP & CONFLICT OF INTEREST

**Contributions:** All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

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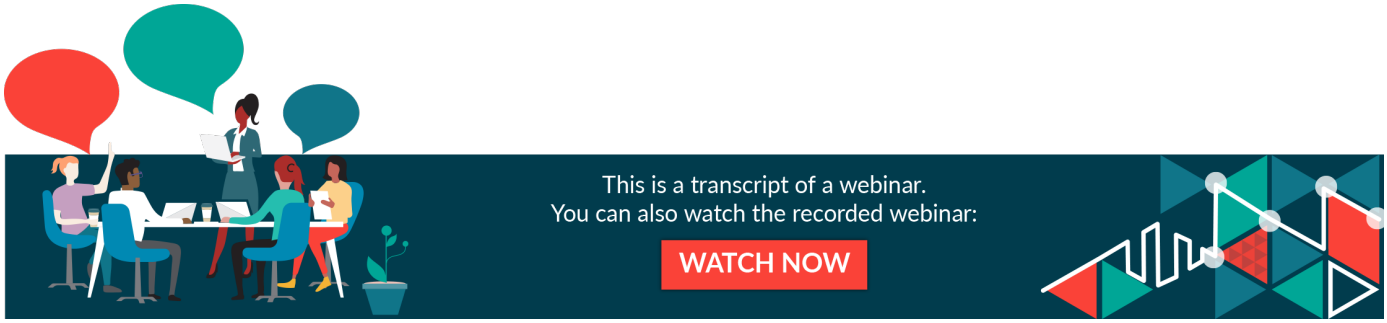



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WEBINAR DIGEST

# Advancing the purification of VSV-G pseudotyped lentiviral vectors by using affinity chromatography

**Pim Hermans,** Head of Ligand Discovery for BioProduction Group, Thermo Fisher Scientific &
**Frank Detmers,** Director of Ligand Application for CaptureSelect™, Thermo Fisher Scientific

**Cell and gene therapy vectors derived from lentivirus (LV) have the ability to integrate the host cell genome, making them effective tools to transduce both dividing and non-dividing cells, and allowing them to provide long-term stable gene expression.**

## CHALLENGES

With a growing pipeline of LV particle-based therapies comes a need for more efficient manufacturing processes that meet the demand for functional LV vectors. However, the following purification challenges need to be overcome in order to reach the purity levels needed for clinical use:

- Both LV particles and extracellular vesicles follow a similar expression route in the cell, which means that separating LV vectors from the large variety of closely-related product forms in the feedstock presents a challenge **(Figure 1)**. Consequently, the production of LV vectors yields a number of variations in terms of both the vector and the exosomes, and analytical assays are required to discriminate between the different particles present in the cell culture feed or purification samples.
- Lentiviral vectors are unstable and require a narrow range of pH, temperature, shear stress, salt concentration, and osmolarity. This makes finding a suitable and efficient purification strategy challenging. As shown in **Figure 2**, current processes report total recoveries of approximately 30% or less.

**Figure 1.** Overview of lentivirus purification challenges and the analytics associated with process development.

Lentivirus feed stock materials derived from human cell lines like HEK293 (also secreting exosomes), will likely contain a variety of product related particle contaminants that are difficult to discriminate:


| Analytical challenges | Important LV prep feature |
|---|---|
| • **How many total particles (TP)?** Particle count (e.g. DLS); P24 ELISA<br>• **How many particles with payload?** RT-qPCR<br>• **How many infectious particles (IP)/transducing units (TU)?** Infectivity assay: FACS/qPCR | • Ratio of TP : IP (TU) - lower the better<br>• Ratio of 200 - 1 in 200 particles infectious |

**Figure 2.** Existing purification methods are inadequate in separating particles with and without a genetic payload.

**Existing purification methods:**

- (Ultra-)centrifugation
- Tangential flow filtration (TFF)
- Anion exchange resins & membranes
- Heparin resins (semi-affinity)

Current multi-step processes: recoveries ~30%

No LV specific affinity chromatography method commercially available

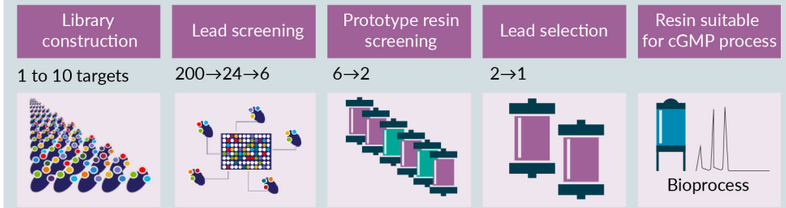
## SOLUTIONS

To overcome the challenges in LV purification, an affinity resin targeting the vesicular stomatitis virus-G (VSV-G) membrane protein was developed using the CaptureSelect™ technology and resin development process.

CaptureSelect™ ligands are:

- Based on single-domain antibody technology
- Developed using an extensive screening technology where final process conditions are already implemented during screening
- Tested for specificity, mild elution conditions, and stability to allow usage in chromatography processes
- Recombinantly expressed in a yeast production process, which is free of animal components
- Resins are developed in a variety of drug development areas such as antibodies, biosimilars, plasma proteins, and viral vectors. **Figure 3** shows the steps involved in the development of a custom CaptureSelect™ affinity resin.

**Figure 3.** CaptureSelectTM affinity resin development.




Lead screening focused on affinity ligand candidates targeting VSV-G as displayed on VSV-G pseudotyped LV vectors and showing efficient release under mild elution conditions[1]
• 0.8 M Arginine at neutral pH was identified as compatible elution buffer for VSV-G LV vectors

[1]Moreira AS, Bezemer S, Faria TQ, *et al*. Implementation of novel affinity ligand for lentiviral vector purification. *Nt. J. Mol. Sci.* 2023; 24(4), 3354.

The CaptureSelect™ Lenti VSV-G affinity matrix is designed to help increase productivity and efficiency in the downstream process of VSV-G pseudotyped lentiviral vectors from suspension culture. It provides gentle elution conditions, based on Arginine, to retain infectivity of the LV particles.

Watch the webinar here

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FASTFACTS

# Efficient, scalable purification of VSV-G lentivirus by novel affinity chromatography

**Frank Detmers**, Director of Ligand Application for CaptureSelect, Thermo Fisher Scientific

**Lentiviral vectors have emerged as a long-term stable gene expression tool for cell and gene therapies. However, large-scale production of purified clinical-grade lentiviral vectors remains a challenge because of the complex feedstock and its sensitivity to changes in temperature, ionic strength, pH, and other environmental factors. This poster presents the chromatography conditions and performance of a recently developed affinity chromatography resin for the purification of lentivirus particles.**

## LENTIVIRUS PURIFICATION CHALLENGES

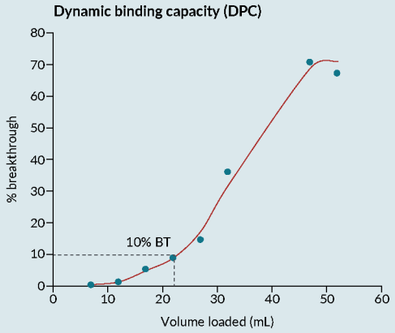
Lentiviral vectors (LVV) have limited stability, requiring a narrow range of pH, temperature, shear stress, salt concentration, and osmolarity. Because of this, traditional methods of purification suffer from difficulties relating to yield, purity, and scalability. With these methods, general recoveries in the field are not higher than 25–30% for the overall process, with a significant part of the losses being in the final filtration step utilizing a sterilizing-grade filter. Thermo Fisher Scientific recently developed an affinity chromatography resin, CaptureSelect™ Lenti VSVG Affinity Matrix, as a solution to these challenges.

## DYNAMIC BINDING CAPACITY OF CAPTURESELECT™ LENTI VESICULAR STOMATITIS VIRUS G (VSV-G) AFFINITY MATRIX

Based on CaptureSelect™ technology, the immobilized ligand is developed to specifically bind to the VSV-G envelope protein present in the vast majority of recombinant lentiviral pseudotypes. Lentivirus produced in HEK-293 cells in suspension is loaded on 0.66 x 3 cm column containing 1 mL of CaptureSelect™ Lenti VSV-G resin, equilibrated in 50 mM HEPES buffer solution, 150 mM NaCl pH 7.5.

As shown in **Figure 1**, 10% breakthrough of the lentivirus particles is reached after loading 24.6 mL of the feed material, resulting in a dynamic binding capacity of the resin of $1x10^{11}$ total particles/ml of resin. $C_0$ is the titer of the feedstock ($3.89 \times 10^9$ particles/mL), and C is the titer measured in the flow through fractions. The 10% breakthrough point is interpolated from the breakthrough curve.

**Figure 1.** Dynamic binding capacity (1 mL column).




**DBC is determined by P24 total particle ELISA**

| Fraction | Volume (mL) | TP/mL | $C/C_0$ | |
|---|---|---|---|---|
| Start | | 3.98E9 | | |
| 1 | 7.1 | 1.55E7 | 0.39 | |
| 2 | 12.1 | 5.24E7 | 1.32 | |
| 3 | 17.1 | 2.14E8 | 5.39 | |
| 4 | 22.1 | 3.53E8 | 8.88 | 24.6 |
| 5 | 27.1 | 5.85E8 | 14.7 | |
| 6 | 32.1 | 1.44E9 | 36.09 | |
| 7 | 47.1 | 2.82E9 | 70.84 | |
| 8 | 52.1 | 2.68E9 | 67.33 | |

- 10% breakthrough ($C/C_0$=10%) estimated from the curve at 24.6 mL loading
- This relates to 9.78E10 total particles/mL resin (=1E11)

Dynamic binding capacity at 10% breakthrough is 1E11 total particles/mL resin

**Figure 2.** Chromatography conditions of the CaptureSelect™ Lenti VSVG affinity matrix.

Endonuclease treatment

↓

LV clarification using microfiltration (0.4 µm)

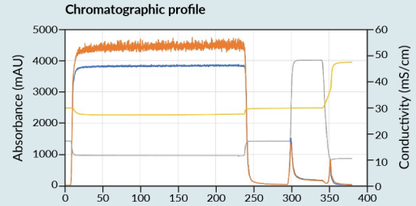
↓

CaptureSelect Lenti VSVG affinity matrix

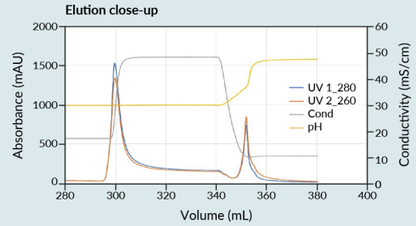
- Column=1.6 × 5 cm (10 mL)
- Flow rate=150 cm/h
- Contact time=2 cm

**Binding/equilibration buffer:** 50 mM HEPES, 150 mM NaCL pH 7.5
**Elution buffer:** 50 mM HEPES, 150 mM NaCL, 0.8 M arginine pH 7.5
**Strip buffer:** 50 mM sodium phosphate pH 12

**Chromatographic profile**




**Elution close-up**






The CaptureSelect Lenti VSVG affinity matrix demonstrates an efficient elution profile

## CHROMATOGRAPHY CONDITIONS

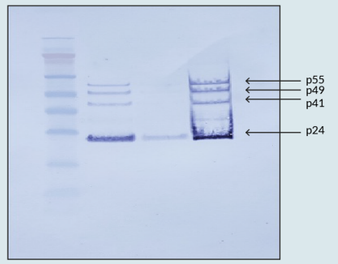
**Figure 2** illustrates that the elution with 50 mM HEPES, 150 mM NaCl, 0.8 M arginine pH 7.5 is efficient and has good compatibility with the enveloped virus particles, resulting in high concentrations of infectious particles in the elution fraction. Depending on the feed and application, optimization of the elution buffer might be needed with adjustments of the arginine concentration, pH, or combinations thereof.

## COMPARISON OF TOTAL PARTICLES TO INFECTIOUS PARTICLE RATIOS

The total concentration of infectious particles increases after purification **(Figure 3)**. Total particles are determined by p24 ELISA and infectious particles are determined through a cell infectivity assay. In the first run, 1 in every 100 particles is infectious in the elution fraction, while in the feedstock it is 1 in every 138 particles. In the second run, this ratio becomes 1 in 165 particles in the feed to 1 in 70 particles in the elution fraction.

**Figure 3.** Concentration of infectious particles in the elution fraction.

**P24–WB pattern**




**Total particle (TP) and infectious particle (IP) ratio**

| Sample | TP/mL | IP/mL | TP/IP ratio |
|---|---|---|---|
| 1. Feed | 1.10E10 | 7.98E7 | 138 |
| 1. Flow through | 3.25E8 | 8.30E5 | 392 |
| 1. Elution | 4.44E10 | 4.42E8 | 100 |
| 2. Feed | 1.11E10 | 9.00E7 | 165 |
| 2. Flow through | 1.28E9 | 5.45E6 | 245 |
| 2. Elution | 2.6E10 | 4.66E8 | 71 |

(n=2)

- The eluted fractions show a more than 5-fold increase of the infectious particle concentration compared to the load

The concentration of infectious particles in the elution fraction has been enriched through purificaton using the Lenti-VSVG resin



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WEBINAR DIGEST

# Simplifying lentiviral downstream processing with a novel affinity resin & robust analytical tools

**Chantelle Gaskin & Suzy Brown**

**Due to its broad tropism and long-term, stable gene expression in non-dividing cells, recombinant lentivirus (LV) has become a vector of choice for many gene-modified cell therapies. The safety and efficacy of LV-based therapies depend greatly on optimized and controlled LV production. Downstream purification of LV particles presents unique challenges, and robust analytics are critical to verify both the recovery and infectivity of the purified product. This poster gives a condensed overview of a new affinity chromatography resin to purify VSV-G pseudotyped LV, as well as qPCR-based genomic and proviral infectious titer assays for analytical use.**

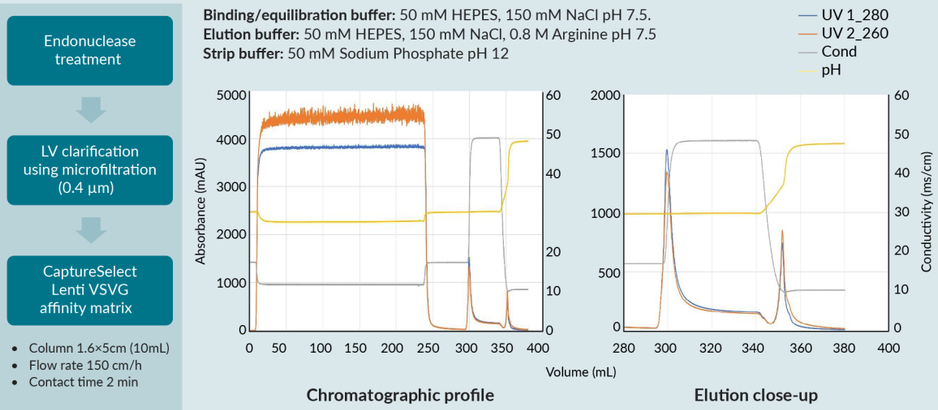
## LENTIVIRAL VECTOR PURIFICATION

The purification of LV vectors is considered as a challenging process. It requires processing within narrow ranges of pH, temperature, conductivity, and shear and existing purification tools often end in low recovery or can affect infectious titers. Affinity chromatography has been the most requested method from the field to overcome these challenges. The newly launched CaptureSelect™ Lenti VSV-G Affinity resin was designed for specificity to VSV-G pseudotyped LV vectors. The resin provides high-level purification in a single step with gentle elution conditions at neutral pH to maximize infectious particle recovery. **Figure 1** shows the recommended chromatography conditions and the elution profile using the affinity resin. The elution was efficient and showed good compatibility with the enveloped virus particles, resulting in high concentrations of infectious particles in the elution fraction. As the process progresses, the total to infectious particle ratio decreases. This results in a >5-fold enrichment of infectious particles with a 50–60% recovery in the column eluate **(Table 1)**.

**Table 1.** Comparison of total particle to infectious particle ratios.

| Sample | TP/mL | IP/mL | TP/IP ratio | Recovery | HCP removal | Total DNA removal |
|---|---|---|---|---|---|---|
| 1. Feed | 1.10×10 | 7.98×7 | 138 | | | |
| 1. Flow through | 3.25×8 | 8.30×5 | 392 | | | |
| 1. Elution | 4.44×10 | 4.42×8 | 100 | 50% | 99% | 80% |
| 2. Feed | 1.11×10 | 9.00×7 | 165 | | | |
| 2. Flow through | 1.28×9 | 5.45×6 | 245 | | | |
| 2. Elution | 2.6×10 | 4.66×8 | 71 | 58% | 97% | 97% |

**Figure 1.** Representative chromatogram using recommended process conditions.




## ANALYTICAL TOOLS FOR PURIFICATION PROCESS DEVELOPMENT

As LV vectors are used to transduce cells, they are an active ingredient in drug substances. Drug substances must be tested for critical quality attributes (CQAs) in-process and at lot release according to US FDA CMC guidance. For LV characterization and integration analysis, Thermo Fisher Scientific has recently introduced two new qPCR assays **(Figure 2)**. The ViralSEQ™ Lentiviral Physical Titer Kit is a one-step real-time RT-qPCR assay for genome-based LV titers, measuring physical titer in viral particles per mL. Additionally, the ViralSEQ™ Lentivirus Proviral DNA Titer Kit is a qPCR assay to measure integrated LV or proviral copies in transduced cells. It can be used to calculate infectious viral titers and vector copy number (VCN). Combined, these two assays provide a convenient method to compare qPCR to qPCR data, for total and infectious titers, as well as for measuring VCN for analytics across the LV workflow. Both assays are designed to provide robust performance and facilitate LV analytics, in-process development, and manufacturing in QC environments.

**Watch the webinar here**

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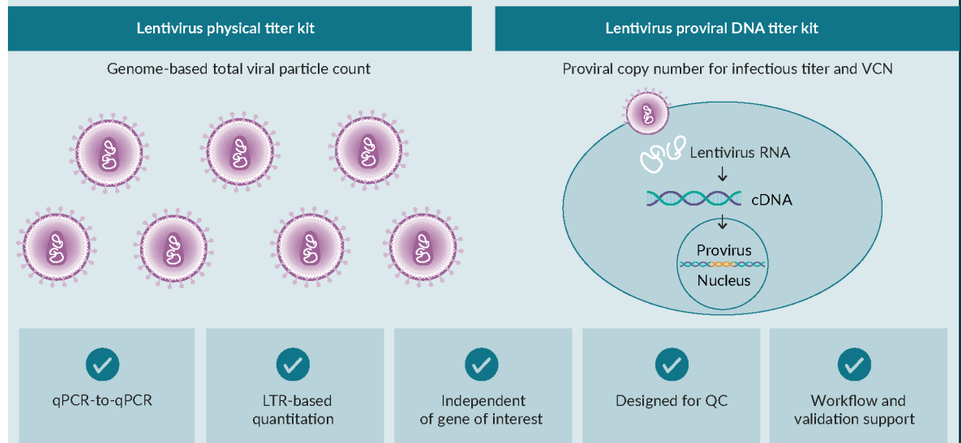
**Figure 2.** Overview of the ViralSEQ Lentivirus titer kits.

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