# High throughput optimization of chromatography steps for viral clearance using retrovirus-like particles (RVLPs)

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## Introduction

- Mammalian cells are vulnerable to infection by known and unknown adventitious agents. In addition, CHO cells, used to produce many biotherapeutics, are known to produce endogenous retrovirus-like particles (RVLPs).
- To ensure patients are safe from adventitious agents and RVLPs, regulatory agencies require demonstration that the purification process removes/inactivates viruses
- Studies using model live viruses must be performed in a laboratory with a suitable biosafety classification, often at a 3rd party facility, which constrains the number of conditions for evaluation
- RVLPs purified from CHO cells are non-infectious and can be quantified by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). A highly concentrated stock (provided by Cygnus Technologies LLC) can be used to study the retroviral clearance capability of downstream unit operations.

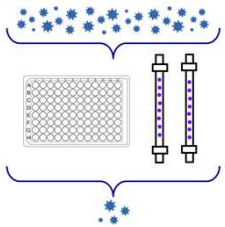


Figure 1: Viral clearance demonstrated via plate-based or bench scale purification processes.

## Objectives

Demonstrate that purified RVLPs coupled with high throughput downstream development tools can enable a cost-effective, convenient evaluation of operating spaces for retroviral clearance by chromatography operations:

1. Determine if purified RVLPs are cleared under similar conditions to the model virus xMuLV in AEX flow through chromatography
2. Determine if high throughput plate-based resin screens can predict performance of bench-scale chromatography runs

## Purification methods

- Purified RVLPs (provided by Cygnus Technologies LLC) were spiked into mock chromatography load at 1% (v/v) to examine the operating space of chromatography unit operations to clear enveloped viruses
- Plate-based screens were conducted using AEX resin, filter plates, and a Tecan liquid handling robot. Adjacent wells were not used to mitigate risk of cross-contamination of RNA signal; using this set-up 24 unique conditions can be evaluated per plate.
- The load sample was incubated in the resin plate for 30 minutes while shaking to achieve equilibrium. The flowthrough was subsequently collected for analysis, as well as the bound material stripped with 1M NaCl.
- Bench-scale chromatography runs were conducted with a packed-bed 0.5 cm ID x 5.3 cm HT column and loaded with the same load:resin volume ratio as the plate-based screen
- The bench-scale flow rate was adjusted to achieve similar residence time to platform residence time for flowthrough polishing
- An RT-qPCR method was used to quantify RVLP in load, flowthrough, and strip samples

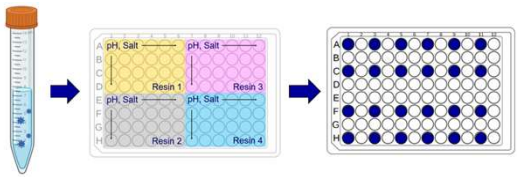
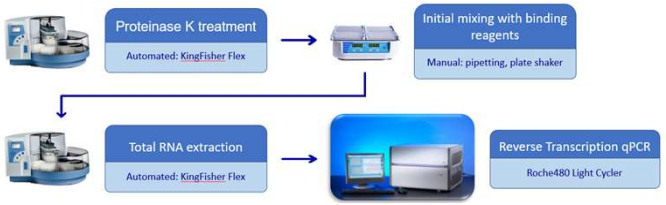



Figure 2: Workflow used for high throughput plate screening, RVLP spiking pattern shown in blue wells.

## Analytical methods

- A high-throughput workflow for the analysis of RVLP content was developed for rapid analysis of in-process samples:




- Total RNA was extracted from samples using the Applied Biosystems™ MagMax™ mirVana™ Total RNA Isolation Kit. Each sample was treated with Proteinase K to digest CHO cell-derived DNA and other proteins, followed by a lysis buffer to release viral RNA.
- Purification of RNA is automated with a KingFisher Flex instrument. Recovered RNA were reverse transcribed, amplified by TaqMan in triplicate, and subsequently quantified.

## Results

- Log Reduction Value ($\mathbf{LRV}$) $= -\log_{10}\frac{c_{\text{flowthrough}}}{c_{\text{load}}}$
- Each method showed consistent RVLP titers in the load samples for different pH and salt combinations, ensuring that trend comparisons for the flowthrough samples can be made accurately. Additionally, analysis of the flowthrough and strip samples provided evidence of 70-80% recoveries of RVLP spike.
- For the plate-based screening, LRV was highest at low salt concentrations. This is consistent with xMuLV and AEX results in literature [1,2]. However, in contrast to these results, pH did not significantly impact LRV.
- At the bench scale, LRV was also highest at low salt concentrations. However, larger LRVs were observed in column runs than the plate-based screening.

| | Bench screening | Plate-based screening |
|---|---|---|
| RVLP Spike | 1% | 1% |
| NaCl (mM) | 0, 200 | 0, 200, 400 |
| pH | 6, 8 | 5, 6, 7, 8 |
| Load Titer (log10 RVLP/mL) | 7.4 ± 0.5 | 7.2 ± 0.1 |

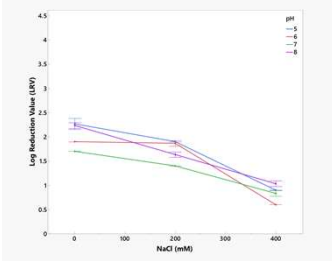



Figure 3: Effect of pH and NaCl concentration on LRV for the plate-based screening.

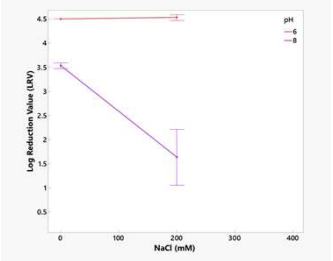



Figure 4: Effect of pH and NaCl concentration on LRV for the bench screening.

[1] Connell-Crowley, Lisa, Elizabeth A. Larimore, and Ron Gillespie. 2013. "Using High Throughput Screening to Define Virus Clearance by Chromatography Resins." Biotechnology and Bioengineering 110 (7): 1984–94. https://doi.org/10.1002/bit.24869.
[2] Strauss, Daniel M., Scott Lute, Kurt Bronson, Gregory S. Blank, Qi Chen, and Bin Yang. 2009. "Removal of Endogenous Retrovirus-like Particles from CHO-Cell Derived Products Using Q Sepharose Fast Flow Chromatography." In Biotechnology Progress, 25:1194–97. https://doi.org/10.1002/btpr.249.

## Discussion

- Previous studies demonstrated clearance of xMuLV at >4 LRV for conditions below 200mM NaCl and above pH 6 [1,2]. Similar results were seen at the bench scale with purified RVLPs, suggesting both RVLPs and xMuLV bind to AEX resin under similar conditions.
- These results utilized chromatography runs with buffer alone to eliminate impact of product and impurities in process streams; future studies will investigate RVLP clearance in presence of mAb products
- Additional studies are needed to investigate the comparability between the plate-based screening and bench scale column runs; the difference between magnitude of clearance observed at low salt conditions (1-2.5 logs) suggests that the plate-based method may require optimization. A time-course experiment could be conducted to determine whether equilibrium binding has been achieved after 30 minutes of incubation under plate-based conditions.
- While the analytical results between the two methods differ slightly, an important consideration is that the trends produced for high throughput screening are consistent with those for bench scale. The contour plot below shows salt concentration is the major determinant of RVLP binding to AEX resin.
- Additional studies with robocolumns will be considered in the future, leveraging a high throughput format for more representative dynamic chromatography experiments

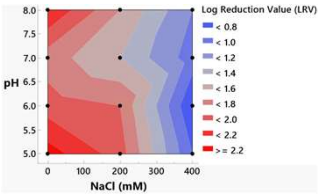



Figure 5: Predictive contour plot for AEX plate-based screening, forecasting LRV trends based on pH and salt concentration.

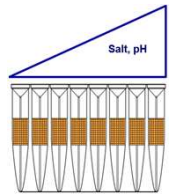



Figure 6: Example of high throughput robocolumn screening study.

## Conclusions

- The ability to optimize downstream processes for viral clearance is often complicated by the need to use live, infectious, model viruses. In-house methods for robust screening of platform conditions combined with a stock viral surrogate allow for a greater number of parameters to be tested in advance of a viral clearance study. Thus, chromatography steps can be optimized for the clearance of RVLPs and other retroviruses during process development prior to execution of formal virus spiking studies.
- At the bench scale, only a single set of run conditions can be tested at a time. A plate-based screening can examine up to 24 different run conditions simultaneously and also uses less RVLP stock solution, serving as a practical predictor for expected trends for the more material- and time-intensive bench scale confirmation runs.
- The use of RVLPs is promising for reducing risk prior to executing GxP viral clearance studies. Initial bench and plate-based screenings show that the clearance of RVLPs trends similarly to the clearance of xMuLV in other studies.
- Beyond initial proof-of-concept results with AEX resin, there is a desire to understand how RVLPs behave in more sophisticated purification environments, examining a wide scope of resins operated in other separation modes, including weak partition, and bind and elute

## Acknowledgements

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