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RESEARCH ARTICLE

High throughput chromatography and analytics can inform viral clearance capabilities during downstream process development for biologics

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Abstract

High throughput process development (HTPD) using liquid handling robotics and RoboColumns is an established methodology in downstream process development to screen chromatography resins and optimize process designs to meet target product profiles. However, HTPD is not yet widely available for use in viral clearance capability of the resin due to a variety of constraints. In the present study, a BSL-1-compatible, non-infectious MVM model, MVM-VLP, was tested for viral clearance assessment with various resin and membrane chromatography operations in a HTPD mode. To detect the MVM-VLP in the high throughput experiments, an electrochemiluminescence immunoassay (ECLIA) assay was developed with up to 5 logs of dynamic range. Storage time suitability of MVM-VLP solutions in various buffer matrices, in the presence or absence of a glycoprotein vaccine candidate, were assessed. Then, MVM-VLP and a test article monoclonal antibody (mAb) were used in a HTPD design that included commercially available ion exchange media chemistries, elucidating a wide variety of viral clearance ability at different operating conditions. The methodologies described herein have the potential to be a part of the process design stage in biologics manufacturing process development, which in turn can reduce risk associated with viral clearance validation studies.

KEYWORDS
antibodies, bioprocess emgineering, downstream processing, high-throughput, vaccines

1 | INTRODUCTION

For biopharmaceuticals produced in mammalian cells, two fundamental, equally important goals arise in downstream process development: first, to produce a drug substance that meets the target product profile, and second, to ensure the product safety requirements set by regulatory agencies, like viral clearance during manufacturing, are achieved.[1–3] To attain the first goal, high throughput screening (HTS) has become an important and effective tool for the rapid evaluation of binding characteristics between chromatography resins and products, as well as product- and process-related impurities.[4] This technique, combined with the use of RoboColumns (100–1000 μL column volume), allows for the exploration of a wide variety of process conditions with minimal material and time requirements. When HTS is performed using these miniature columns in conjunction with a liquid handling device, the results prove scalable and predictive of lab-scale processes, primarily due to representative packed bed characteristics

Abbreviations: AEX, anion exchange; BSL, biosafety level; CEX, cation exchange; CHO, Chinese hamster ovary; ECLIA, electrochemiluminescence immunoassay; HTPD, high throughput process development; HTRS, high throughput resin screen; HTS, high throughput screening; LRV, log reduction value; MVM, minute virus of mice; SGBE, salt-gradient bind and elute; VLP, virus like particle

and well-controlled flow rates.[5–8] Thus, HTS has become an invaluable tool for identifying and selecting optimal process conditions early in the development timeline.

Despite the numerous benefits HTS has shown to date, it remains a challenge to use this platform to predict the viral clearance capabilities of chromatography resins. Typically, viral clearance validation is accomplished through "spiking studies" whereby model mammalian viruses (e.g. MVM) are artificially introduced ("spiked") into biopharmaceutical material and subsequently removed via scaled-down purification steps such as chromatography and nanofiltration.[9–12] Recently, several reported studies have leveraged HTS to inform viral clearance capabilities by spiking samples with mammalian virus or bacteriophage.[13,14,15] However, spiking studies that require live virus, whether in HTS or using scale-down models, present numerous challenges, including the necessity of BSL-2 laboratories, safety and feasibility concerns with infectious virus, low-throughput and time-consuming data analysis, and significant cost.[16]

Thus, downstream processes are commonly developed and optimized while relying on historical published data, and viral clearance validation is then performed for each product prior to regulatory submission and evaluation. Unfortunately, the lack of process-specific data early in the development timeline increases the risk of validation failure, which can lead to delays and increased associated costs. This risk, while mitigated by the use of platform processes, can be substantial for novel clinical vaccine candidates that lack industry-standard chromatography methods or platform data from validation studies with similar molecules, e.g. monoclonal antibodies.

It has been previously postulated that a Virus Like Particle (VLP), engineered to represent the physicochemical properties of Minute Virus of Mice (MVM), could serve as an accurate surrogate for predicting MVM removal during early process development or characterization studies by chromatographic and nanofiltration modes of separation.[17–21] By adding this BSL-1 compatible MVM-VLP into in-process feed stream and processing it through a separation technique, Log Reduction Values (LRV) can be determined; however, as MVM-VLP is non-infectious and does not contain internal nucleic acid, infectivity and qPCR methods of detection are unable to provide particle concentration values. In previously reported studies, Immuno-qPCR techniques were established and utilized to quantify the particle concentration of samples generated during nanofiltration studies Although effective, the Immuno-qPCR assay was limited by sample throughout and, at the time of this study, dynamic range.[18–21] To address these disadvantages, we sought to develop a electrochemiluminescence immunoassay (ECLIA) method for measuring the removal of > 4 $\log_{10}$ MVM-VLP.

Herein, we describe the use of MVM-VLP in chromatography screening studies that are performed in a standard BSL-1 development laboratory environment and analyzed by a high-throughput analytical method. These studies provide an estimate of viral clearance capability early in the preclinical development process, prior to viral clearance and scale down model validation studies, reducing the risk of wasted time and resources as a result of failed viral clearance validation. First, we developed a high-throughput ECLIA method with a dynamic range of 5 $\log_{10}$ MVM-VLP. This method was then utilized for MVM-VLP/buffer matrices stability studies, proof of concept chromatographic separations, and finally the application of high-throughput screening of MVM-VLP removal by a variety of ion-exchange resins. Taken together, these methodologies can significantly de-risk viral clearance validation as clinical manufacturing process parameters will be informed by ample clearance data that is comparable in quality and approaches the sensitivity to that of conventional infectivity analysis.

2 | MATERIALS AND METHODS

2.1 | MVM-VLP stock and detection antibody

MVM-VLP was produced by the recombinant expression of MVM's major capsid protein, VP2 and purified according to previously published methods.[18] Negative staining Transmission Electron Microscopy (TEM) was utilized to determine MVM-VLP titer. A stock solution of MVM-VLP was prepared by diluting the preparation to 1.0×10^{12} particles per milliliter with a proprietary formulation buffer. Anti-MVM-VLP detection monoclonal antibodies (mAb) were engineered, produced, and purified according to standard hybridoma procedures[22,23] at Bluepoint Bioscience, Ijamsville, MD.

2.2 | Test articles

The mAb test article employed for all chromatography experiments (section 2.5) came from developmental feedstock generated during the production of a therapeutic monoclonal antibody (mAb) with a pI of 8.27, expressed in a CHO cell line. To generate this test article, clarified cell culture harvest material was purified by Protein A chromatography, held at low-pH for viral inactivation, neutralized and polished via weak anion-exchange interaction chromatography. The preparation was then buffer exchanged to pH 5.8 by ultrafiltration/diafiltration (UF/DF).

The glycoprotein vaccine candidate (~ 350 kDa) test article assessed in the MVM-VLP Buffer Matrix and Storage-time Suitability study (study 2.4) was produced in a CHO cell line and purified by conventional resin chromatography.[24]

2.3 | Electrochemiluminescence immunoassay (ECLIA)

Microtiter assay plates were coated with purified anti-MVM-VLP mAb according to standard protocols. To perform the ECLIA, wells containing the immobilized detection mAb were incubated with dilution series standards or unknown sample for 60 minutes with shaking at 800 rpm. All assay steps were performed at room temperature unless otherwise stated. All wells were washed with PBST (Phosphate Buffered Saline with 0.05% Tween 20) and incubated with labeled secondary antibody for 60 minutes with shaking at 800 rpm. Wells were then washed and incubated in Read Buffer (MSD Read buffer T) for no more

than 2 minutes. Binding of the captured MVM-VLP within each well was then measured by a Sector S 600 instrument (Meso Scale Diagnostics, Rockville MD). A 5PL non-linear regression curve was fit from the eight-point standard dilution series of VLP ranging in concentration from 1.0×10^{12} to 1.0×10^{5} VLP mL^{-1}. Graph Pad Prism 7.0 (Prism) was used to determine the statistical qualities of the standard curve as well as to interpolate the values of unknown samples to generate their relative binding concentration.

2.4 | MVM-VLP buffer matrix and storage-time suitability

A study was designed wherein MVM-VLP was spiked into material containing vaccine (a purified glycoprotein of ~ 350 kDa) or buffer (matrix of pH 6.5/8.5, [NaCl] of 100/500 mM) at concentrations of 1×10^{7}, 1×10^{8}, 1×10^{9} and 1×10^{10} MVM-VLP mL^{-1} and held for 2 days at 4°C. At time points of 0, 4, 24 and 48 h, samples were frozen at -80°C. All samples were then thawed and analyzed for MVM-VLP concentration according to ECLIA. Preliminary studies were performed with the purified glycoprotein vaccine candidate as well as a mAb (data not shown). Although all other experiments described herein were performed with the mAb test article, the buffer matrix and storage time stability study was performed with the vaccine candidate due to the existing availability of comparable dataset for a mAb.[19]

2.5 | Binding studies

A Tecan Freedom EVO200 bioprocessing system, equipped with an 8-channel fixed-tip pipettor arm for liquid handling, a robotic arm (RoMa) for plate manipulation, and an integrated UV-vis spectrophotometer (Infinite 200 PRO) was used for high throughput chromatography. All Columns used in this study were OPUS 100 μL RoboColumns. Two of the three studies utilized column hardware, allowing control over residence time, a scale-independent parameter in chromatography process development.

The third study was performed using two 96-well plates containing either STIC PA or Sartobind Q membranes (Sartorius, item number: 99STPA42GC−−D and 99IEXQ42GC−−V, respectively). In contrast to controlled flow rate liquid dispense used with RoboColumns in the first two studies, the mobile phase in the third study is passed through the filter plate via centrifugation. Thus, this membrane-based study does not allow for analysis of residence-time dependent binding kinetics but does provide basic binding data.

2.5.1 | Flow through (FT) vs. salt gradient bind and elute (SG-BE) comparative studies

First, a preliminary Anion Exchange Chromatography (AEX) study was conducted with RoboColumns containing 100 μL of Q Sepharose FF (Q SFF) resin (GE, Piscataway, NJ) in which the clearance of MVM-VLP-spiked test article load was assessed in flow through mode. RoboColumns were equilibrated with 500 μL of various equilibration buffers (pH 6.5, 7.5 and 8.5; [NaCl] of 10, 100, 200, 300, 400 and 500 mM). For each condition, 500 μL of pH- and conductivity-adjusted test article was spiked to 1.0×10^{11} MVM-VLP mL^{-1} and loaded onto the equilibrated RoboColumn. The unbound flow through fraction was collected into a deep well plate and stored at room temperature until analysis, along with the load samples at each pH and salt condition. ECLIA was used to quantify the MVM-VLP in the load samples and unbound flow through fractions. Log Reduction Values (LRV) were calculated from the average of two sets of samples per experiment.

A comparative experiment was then conducted in which the Q SFF chromatography was operated with a salt gradient, bind-elute strategy. Robocolumns were equilibrated with 500 μL of several equilibration buffers (pH 6.5, 7.5, 8.5; [NaCl] of 10 mM). Samples of the test article were adjusted to each equilibration condition, spiked to 1.0×10^{11} MVM-VLP mL^{-1}, and loaded onto the equilibrated RoboColumns. Unbound flow through materials were collected into a deep well plate. Each RoboColumn was then subjected to a series of increasing salt concentration solutions ([NaCl] of 100, 150, 200, 250, 300, 400, and 1000 mM). The load samples and gradient fractions collected during each round were collected and stored at room temperature until ECLIA analysis. While calculating LRV, "Pool" MVM-VLP concentration values were determined from a total running sum of detected particles in all prior fractions. For example, when determining the number of particles present in the pH 6.5/200 mM NaCl experiment, the sum of all particles detected in the flow through, chase, 10, 100, and 150 mM gradient fractions was calculated in comparison to load MVM-VLP concentration. LRVs obtained from each condition and among each throughput strategy were compared.

2.5.2 | High throughput resin screens

High throughput AEX and Cation Exchange Chromatography (CEX) resin screens were then conducted using RoboColumns in salt gradient bind-elute mode. 100 μL RoboColumns containing the resins listed in Table 1 were equilibrated with 500 uL of the specified condition. For each resin and pH condition, 500 μL of pH-adjusted test article load material was spiked to 1.0×10^{11} MVM-VLP mL^{-1} and loaded on to the equilibrated RoboColumn. The unbound flow through fractions were collected into a deep well plate. A series of increasing salt concentration solutions were then transferred to each RoboColumn and collected separately. The load samples and all fractions were collected and stored at room temperature until ECLIA analysis. LRVs were calculated as described above.

2.5.3 | Membrane chromatography studies

High throughput MVM-VLP spiking experiments were performed on 96 well Sartobind STIC-PA and Sartobind Q anion exchange

TABLE 1 Anion and cation exchange resin screen parameters

Chemistry	Resin	Manufacturer	Equilibration Condition	Gradient Steps [NaCl] (mM)
AEX	Toyopearl DEAE 650 M	Tosoh, Germany	pH: 6.5, 7.5, or 8.5 [NaCl]: 10 mM	100, 150, 200, 250, 300, 400, and 1000
	Toyopearl NH2-750F			
	POROS 50D	ThermoFisher, Waltham, MA		
	POROS 50PI			
	POROS 50HQ			
	Q Sepharose FF	GE, Picastaway, NJ		
	Fractogel EMD TMAE HiCap	Millipore Sigma, Burlington, MA		
	Fractogel EMD DEAE (M)			
CEX	POROS XS	ThermoFisher, Waltham, MA	pH: 5.5 or 6.5 [NaCl]: 10 mM	50, 100, 150, 200, 250, 300, 350, 500, and 1000
	POROS 50HS			
	SP Sepharose FF	GE, Picastaway, NJ		
	Nuvia S	BioRad, Hercules, CA		
	Macro-Prep CM			
	Eshmuno S	Millipore Sigma, Burlington, MA		
	Toyopearl GigaCap CM-650 M	Tosoh, Germany		
	CM Ceramic HyperD F	Pall Corporation, Port Washington, NY		

This table details the chromatography chemistries, manufacturers, and operating pH/NaCl conditions tested in the AEX and CEX resin screens.

membranes (Sartorius, Germany). These experiments were performed in salt gradient bind-elute mode as described above. In brief, each membrane was equilibrated with 500 μL of buffers conditioned to pH 6.5, 7.5 or 8.5 containing 10 mM NaCl. Following equilibration, 500 μL of MVM-VLP-spiked (1.0×10^{11} MVM-VLP mL^{-1}), pH-adjusted, test article load material was loaded onto the equilibrated membrane. A series of increasing salt concentration solutions were then transferred to each membrane (10, 50, 100, 150, 200, 250, 300 and 500 mM). Each fraction was passed through the membrane via centrifugation, collected separately, and stored at -80°C until ECLIA analysis. LRVs were calculated as described above.

3 | RESULTS

3.1 | ECLIA performance

The developed ECLIA method supported the experimental needs of high-throughput testing, low sample volume requirements, and performed with a wide dynamic range of reporting. The assay took 3 h, required only 30 μL of sample volume, and both the assay design and data acquisition were compatible with automation. This assay was able to demonstrate a dynamic range of nearly 5 $\log_{10}$, enabling LRVs of $\geq$ 4.85 $\log_{10}$ to be determined. All r-squares were greater than 0.98 and all %CVs less than 30%.

3.2 | MVM-VLP buffer matrix and storage-time suitability

A time course study was designed and executed to assess the impact of storage time and buffer matrix on the quantification of MVM-VLP. The results (Figure 1) indicate that in the absence of test article, at the lowest target MVM-VLP concentration (1.0×10^8 MVM-VLP mL^{-1}) there were significant decreases in the measured concentration of particles as a function of time. Generally, in the presence of test article, no such trends occurred across the time points regardless of MVM-VLP concentration, NaCl concentration, or pH. The overall measurement consistency of MVM-VLP spiked to a target concentration of 10^{10} MVM-VLP mL^{-1} from 0 to 48 h for samples without test article suggest that the higher concentration of particles negated the effect seen at the lower concentrations.

3.3 | Chromatography studies

3.3.1 | Flow through (FT) versus salt gradient bind and elute (SG-BE) comparative studies

To establish a strategy for further high-throughput experiments and to verify overall proof of concept, Q S-FF flow-through (FT) and salt gradient bind-elute (SG-BE) experiments were conducted. LRVs from each set of experiments were determined and compared in Table 2. The

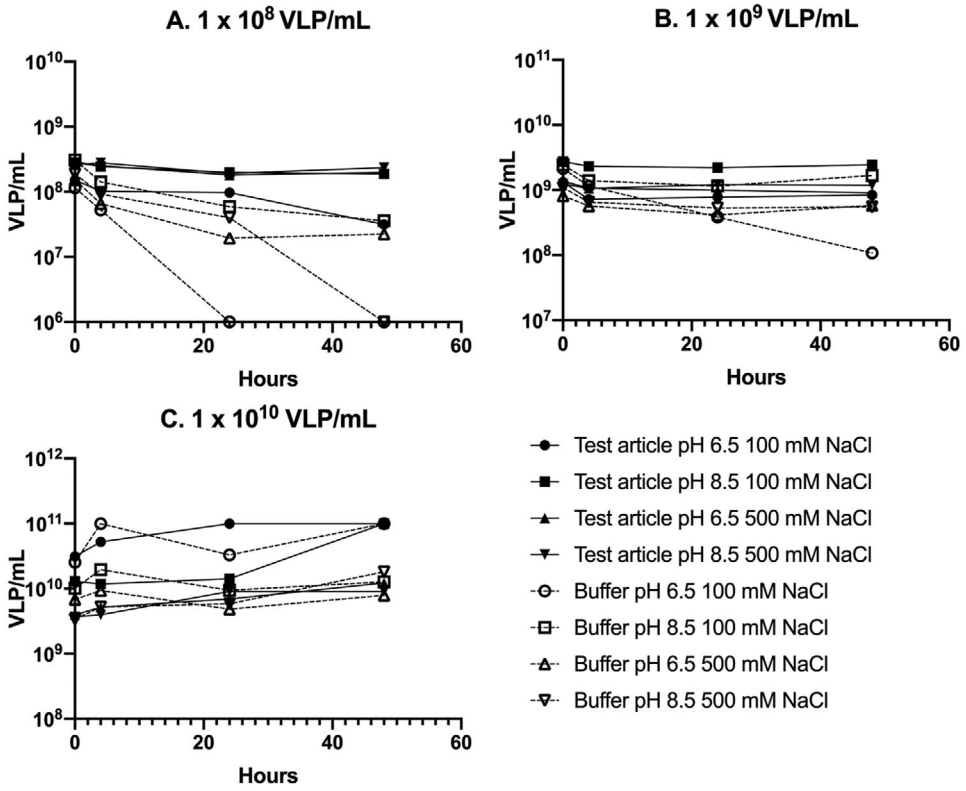

FIGURE 1 Buffer matrix and storage-time suitability study results. This figure illustrates the change in measured MVM-VLP concentration (each data point, n = 1) over time for three different starting concentrations and eight conditions for each. Starting concentrations of 1×10^8, 1×10^9, and 1×10^{10} VLP/mL were assessed with and without a 350 kDa glycoprotein vaccine candidate test article and in various pH and [NaCl] conditions. Samples were measured at 0, 4, 24, and 48 hours

results indicate that full clearance of MVM-VLP, as indicated by "≥", was achieved at NaCl concentrations up to 150 mM, regardless of pH or experimental mode. At NaCl concentrations of 200 and 250 mM, a decrease in clearance of MVM-VLP was seen at a pH of 6.5 for both FT and SG-BE modes, while full or partial clearance of MVM-VLP was achieved in higher pH samples. At NaCl concentrations higher than 250 mM, significant breakthrough of MVM-VLP occurred across all conditions tested and modes of operation, as indicated by a complete lack of LRV in these samples.

3.3.2 | HTRS AEX study

High throughput resin screening (HTRS) of AEX chromatography resins was conducted in salt gradient bind-elute mode. Eight AEX resins at three pH equilibration/load conditions were examined in singlet. Table 3 summarizes the LRV results from each experiment graphically presents LRV clearance as a function of pH and elution salt concentration. Overall, the results demonstrated the wide variability in pH and salt tolerance for MVM-VLP clearance through the anion exchange resins tested. In addition, clearance of MVM-VLP by Q SFF correlated well with the previous SG-BE results reported in Table 2.

3.3.3 | HTRS CEX study

Similarly, high throughput CEX resin screening was conducted in salt gradient bind-elute mode. Eight resins at two pH equilibration/load conditions were examined in singlet. Table 4 summarizes the LRV values from each experiment. These results demonstrate the variability among the negatively charged resins to retain the positively charged MVM-VLP at lower pH (5.5) and flow through/chase NaCl concentration (10 mM NaCl). Nuvia S and Poros 50HS were shown to fully retain MVM-VLP at these conditions while others were seen to have moderate to poor retention. After applying 50 mM NaCl, a majority of the MVM-VLP still bound to Nuvia S and Poros 50HS eluted off into the collection fraction, decreasing the calculated LRV's to 1.09 and 1.83 respectively. After applying 100 mM NaCl, nearly all bound MVM-VLP was eluted and LRV's of ≤ 1.0 were achieved for all resins.

3.3.4 | Membrane chromatography studies

Sartobind STIC-PA and Sartobind Q AEX membranes were also screened for MVM-VLP clearance in salt gradient bind-elute mode using 96-well plates. The results from these experiments (Table 5) showed that STIC-PA has a significantly higher salt tolerance for

TABLE 2 Salt-gradient bind and elute (SG-BE) results compared with flow through (FT) chromatography results

Mode	NaCl mM	pH		
		6.5	7.5	8.5
FT	10	≥ 4.85	≥ 4.85	≥ 4.85
SG-BE		≥ 4.85	≥ 4.85	≥ 4.85
FT	100	≥ 4.85	≥ 4.85	≥ 4.85
SG-BE		≥ 4.70	≥ 4.70	≥ 4.41
FT	150	≥ 4.59	≥ 4.59	≥ 4.34
SG-BE		ND	ND	ND
FT	200	2.15	≥ 4.85	≥ 4.35
SG-BE		2.14	≥ 4.49	≥ 4.28
FT	250	ND	ND	ND
SG-BE		0.19	2.64	3.94
FT	300	0.55	0.57	1.12
SG-BE		-0.07	-0.07	1.30
FT	400	0.68	0.72	0.69
SG-BE		-0.08	-0.23	0.38
FT	500	1.01	0.62	0.78
SG-BE		ND	ND	ND
FT	1000	ND	ND	ND
SG-BE		-0.08	-0.23	0.37

Mean LRVs (n = 2) for each [NaCl] and pH condition are displayed for SG-BE and FT modes on Q S FF resin.
LRVs are color coded where green represents better MVM-VLP clearance and red indicates low or no MVM-VLP clearance.

MVM-VLP removal than Sartobind Q with the ability to remove > 3.5 $\log_{10}$ MVM-VLP up to 500 mM NaCl.

4 | DISCUSSION

A fundamental, yet critical task of downstream process development is the proper selection of the chromatography process that will be relied upon to accomplish the goals of the purification process; specifically, the production of a highly pure drug substance while adhering to product safety guidelines set forth by regulatory agencies, including proving sufficient viral clearance. Traditionally, the analysis of viral clearance requires significant capital investment, complicated logistical management, and specialized laboratories and personnel. Although some illuminating high-throughput viral clearance studies have been reported,[13,14,25] the challenges of working with infectious virus eliminate this possibility for most process development laboratories. In contrast, the implementation of high throughput screening techniques in typical BSL-1 development labs has greatly increased efficiency and sample throughput in process development. Using batch format 96-well plates or RoboColumns containing 100 μL of various resins, binding characteristics can quickly be evaluated over a wide range of parameters. Rich data sets from such experiments can form the basis for further development of resins or membranes.

In the present study, a non-infectious MVM surrogate (MVM-VLP) was used as a model for MVM clearance for a variety of anion and cation exchange products in high throughput screening format. Before MVM-VLP was evaluated in combination with vaccine feed stream in chromatography experiments, a rapid electrochemiluminescence immunoassay (ECLIA) was developed. The assay as run in these experiments can demonstrate 4–5 logs of MVM-VLP clearance.

Then, a time course MVM-VLP quantitation study was performed, demonstrating that the presence of a ~350 kDa glycoprotein vaccine candidate within a sample significantly stabilized MVM-VLP quantitation via ECLIA over time. In the absence of the glycoprotein, there were significant decreases in measured MVM-VLP concentration between the 24- and 48-h time points for each initial target concentration of MVM-VLP, except at 1.0×10^{10} particles per milliliter. In the presence of glycoprotein, however, no such trend occurred at any of the initial target MVM-VLP concentrations tested, regardless of NaCl concentration or pH. This phenomenon has been previously reported with a mAb test article[19] and might be explained if the proteins serve as a surfactant, effectively coating the sample tube and maintaining a high percentage of the MVM-VLP in free, accessible solution. At higher concentrations of MVM-VLP in the absence of the test article, perhaps the total amount required to "coat" the surface of the tube is still a relatively low percentage of the total MVM-VLP present and thereby no downward trending effect can be measured. Conversely, at lower concentrations, the total amount of MVM-VLP required to "coat" the surface is high, relative to the total amount of MVM-VLP in the sample and thus a decrease in concentration is noticed. Nonetheless, this initial study provided critical information on sampling logistics as not all fractions would necessarily contain test article. Three strategies were considered: first, immediately analyzing all samples after the completion of an experiment as data supported stable particle quantitation for > 4 h, second, spiking the collection vessels with a carrier protein such as BSA, and third, freezing samples at -80°C. For the subsequent high throughput chromatography studies, the first strategy was available and chosen.

Prior to this study, a MVM-VLP surrogate model had never been utilized to predict viral clearance at a scale appropriate for high-throughput testing. Thus, it was essential to prove that clearance

TABLE 3 Anion exchange resin screen results

Resin	Load FT	mM NaCl								
		50	100	150	200	250	300	350	500	1000
Toyopearl DEAE-650M pH 6.5	4.80	4.66	1.16	0.45	0.45	0.44	0.44	0.44	0.44	0.44
Toyopearl DEAE-650M pH 7.5	4.80	4.66	4.55	1.16	0.73	0.72	0.72	0.72	0.72	0.72
Toyopearl DEAE-650M pH 8.5	4.80	4.66	4.55	2.01	0.77	0.74	0.73	0.73	0.73	0.73
Poros 50D pH 6.5	4.80	4.66	4.09	0.51	0.48	0.47	0.47	0.47	0.46	0.46
Poros 50D pH 7.5	4.80	4.66	4.55	3.18	0.94	0.86	0.84	0.83	0.82	0.82
Poros 50D pH 8.5	4.80	4.66	4.55	1.19	0.94	0.87	0.87	0.86	0.86	0.86
Poros 50PI pH 6.5	2.60	2.43	2.42	2.42	2.42	2.41	2.40	1.16	0.82	0.80
Poros 50PI pH 7.5	4.80	4.66	4.55	4.47	4.40	4.34	4.28	3.95	0.73	0.71
Poros 50PI pH 8.5	4.80	4.66	4.55	4.47	4.03	3.81	3.67	1.95	0.85	0.83
Poros 50HQ pH 6.5	2.92	2.82	2.82	2.73	0.66	0.65	0.65	0.64	0.64	0.64
Poros 50HQ pH 7.5	4.80	4.12	4.09	4.06	2.08	0.95	0.94	0.93	0.93	0.93
Poros 50HQ pH 8.5	4.80	4.66	4.55	4.47	4.40	0.97	0.95	0.94	0.94	0.94
GE Q Sepharose FF pH 6.5	4.80	4.66	4.55	4.47	2.31	0.64	0.57	0.57	0.57	0.57
GE Q Sepharose FF pH 7.5	4.80	4.66	4.55	4.47	4.40	2.30	1.00	0.76	0.74	0.73
GE Q Sepharose FF pH 8.5	4.80	4.66	4.55	4.06	4.03	3.81	1.37	0.93	0.84	0.83
Fractogel EMD TMAE HiCAP pH 6.5	4.80	4.66	4.55	0.36	0.30	0.30	0.30	0.30	0.30	0.30
Fractogel EMD TMAE HiCAP pH 7.5	4.80	4.66	4.55	4.47	0.57	0.49	0.48	0.48	0.48	0.48
Fractogel EMD TMAE HiCAP pH 8.5	4.80	4.66	4.55	4.47	1.14	0.81	0.80	0.80	0.80	0.79
Fractogel EMD DEAE (M) pH 6.5	4.80	4.66	4.55	2.04	0.69	0.68	0.68	0.67	0.67	0.66
Fractogel EMD DEAE (M) pH 7.5	4.80	4.66	4.55	4.47	0.98	0.58	0.57	0.56	0.56	0.56
Fractogel EMD DEAE (M) pH 8.5	4.80	4.66	4.55	1.09	0.79	0.78	0.77	0.77	0.77	0.77
Toyopearl NH2-750F pH 6.5	4.80	4.66	4.55	4.47	4.40	3.37	3.32	3.31	3.26	1.27
Toyopearl NH2-750F pH 7.5	4.80	4.66	4.55	4.47	4.40	4.34	4.28	4.28	4.23	1.23
Toyopearl NH2-750F pH 8.5	3.80	3.78	3.76	3.75	3.73	3.61	3.60	3.59	3.58	1.08

Anion exchange resins were evaluated at pH 6.5, 7.5, and 8.5 in SG-BE mode.
Mean LRVs (n = 2) for each condition are displayed, with green indicating higher LRV and red indicating low or no MVM-VLP clearance.

TABLE 4 Cation exchange resin screen results

Resin	FT/Chase (10 mM NaCl)	mM NaCl								
		50	100	150	200	250	300	350	500	1000
Poros XS pH 5.5	3.80	1.57	0.80	0.77	0.75	0.74	0.74	0.73	0.73	0.72
Poros XS pH 6.5	2.74	1.68	0.49	0.47	0.46	0.46	0.46	0.46	0.46	0.46
SP Sepharose FF pH 5.5	3.03	0.58	0.48	0.47	0.47	0.47	0.47	0.47	0.47	0.46
SP Sepharose FF pH 6.5	1.66	1.35	0.55	0.54	0.53	0.53	0.53	0.53	0.53	0.53
Nuvia S pH 5.5	4.80	1.09	0.99	0.97	0.95	0.94	0.93	0.93	0.92	0.87
Nuvia S pH 6.5	1.35	1.06	0.67	0.64	0.63	0.63	0.63	0.63	0.63	0.63
Eshmuno S pH 5.5	1.95	1.01	0.95	0.91	0.88	0.86	0.85	0.84	0.82	0.77
Eshmuno S pH 6.5	1.22	0.76	0.70	0.69	0.69	0.69	0.69	0.68	0.68	0.68
Toyopearl GigaCap CM-650M pH 5.5	2.54	0.69	0.55	0.54	0.54	0.54	0.53	0.53	0.53	0.52
Toyopearl GigaCap CM-650M pH 6.5	1.23	1.12	0.52	0.50	0.50	0.50	0.50	0.50	0.50	0.50
CM ceramic HyperD F pH 5.5	2.29	1.41	0.83	0.80	0.79	0.78	0.78	0.78	0.78	0.77
CM ceramic HyperD F pH 6.5	2.81	1.98	1.02	0.98	0.97	0.96	0.96	0.96	0.96	0.95
Macro-Prep CM pH 5.5	0.94	0.72	0.70	0.70	0.69	0.69	0.69	0.69	0.69	0.68
Macro-Prep CM pH 6.5	1.46	0.51	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Poros 50HS pH 5.5	4.80	1.83	0.40	0.39	0.39	0.38	0.38	0.38	0.38	0.38
Poros 50HS pH 6.5	2.61	2.36	-0.08	-0.09	-0.09	-0.09	-0.09	-0.09	-0.09	-0.09

Cation exchange resins were evaluated at pH 5.5 and 6.5 in SG-BE mode.
Mean LRVs (n = 2) for each condition are displayed, with green indicating higher LRV and red indicating low or no MVM-VLP clearance.

data could be generated when utilizing 100 μL RoboColumns and a liquid handling system. An initial set of flow-through experiments with Q SFF was able to demonstrate ≥4.85 $\log_{10}$ clearance of MVM-VLP. Furthermore, the clearance of MVM-VLP was observed to be dependent on load pH and NaCl concentration, encountering a significant drop off in clearance between 100 and 200 mM NaCl at a of pH 6.5 and between 200 and 300 mM NaCl in the samples with a pH of 7.5 and 8.5. These results align with expected trends for MVM clearance and previously reported MVM-VLP clearance data with packed Q SFF resin.[19,20] The close agreement with anticipated MVM clearance results demonstrate both the ability to use MVM-VLP with the high throughput system, as well as the utility of the particles themselves.

The Q SFF salt gradient, bind-elute (SG-BE) experiments sought to demonstrate the equivalence of operating in a bind/elute mode vs. the FT mode established in the first proof of concept study. Overall, results demonstrated comparable LRV agreement between the two modes of operation. The maximum clearance that could be measured was achieved at each pH up to a salt concentration of 150 mM for each mode of operation. At a pH of 6.5, LRVs in both modes decreased steeply at 200 mM NaCl for FT vs. SG-BE – from > 4 to 2.15 and 2.14, respectively. At pH of 7.5, LRVs of < 1.0 were determined for each mode at salt concentrations above 300 mM while at pH 8.5, 1.12 and 1.30 $\log_{10}$ reductions were seen for FT vs. SG-BE, respectively at 300 mM salt. The ability to capture additional NaCl gradient elution fractions in the region near LRV drop-off (250 mM) enabled an

TABLE 5 Membrane chromatography screen results

pH	Membrane	mM NaCl							
		10	50	100	150	200	250	300	500
6.5	STIC PA	4.20	4.10	4.03	3.90	3.85	3.81	3.78	3.74
	Sartobind Q	3.65	3.58	3.55	3.52	3.50	2.55	0.79	0.75
7.5	STIC PA	3.70	3.68	3.66	3.64	3.62	3.60	3.59	3.57
	Sartobind Q	3.14	3.14	3.13	3.13	3.12	3.11	2.26	0.81
8.5	STIC PA	3.70	3.68	3.66	3.64	3.62	3.60	3.59	3.57
	Sartobind Q	2.94	2.94	2.94	2.93	2.93	2.93	2.92	0.91

STIC PA and Sartobind Q AEX membranes were assessed at pH 6.5, 7.5, and 8.5.
Mean LRVs (n = 2) for each condition are displayed, with green indicating higher LRV and red indicating low or no MVM-VLP clearance.

enhanced granularity of MVM clearance prediction as compared to FT mode.

A direct comparison of SG-BE LRV to FT LRV was made possible by calculating LRV based on the cumulative total of MVM-VLP detected in all prior NaCl concentration. This approach required the assumption that an LLOQ value obtained at a given elution step represented an actual number of particles present. Therefore, the maximum demonstrable clearance decreased from ≥ 4.85 $\log_{10}$ with each step even when maximum clearance for that step was achieved. Regardless, there was agreement with the flow-through LRV results. Proving equivalence between SG-BE and FT modes allowed the subsequent experiments to minimize time and material usage. Chromatography load materials were prepared and spiked with MVM-VLP in three different pH conditions for SG-BE mode, compared to eighteen individual conditions that would be necessary for FT mode.

Utilizing the described SG-BE method, multiple resins were screened and fractions collected in one 96 well deep-well plate. The first HTRS examined 8 AEX resins at 3 pH conditions and 10 NaCl conditions in singlet with a repeat of Q SFF as a comparative control. The LRV data derived from this experiment confirmed a good comparability of the Q SFF data from the proof of concept run and demonstrated the overall wide variability in pH and salt tolerance among anion exchange resins. Another HTRS was performed for CEX resins. The results demonstrate a modest variability among resins for retaining MVM-VLP at various pH and conductivity; however, LRVs were not as robust as seen in the AEX resin screen.

Lastly, we examined the effect of pH and NaCl concentration on MVM-VLP clearance via AEX membranes. As expected based on manufacturer claims, STIC-PA showed significantly higher salt tolerance than Sartobind Q membranes. In fact, STIC-PA demonstrated ≥ 3 $\log_{10}$ clearance of MVM-VLP in conditions up to 500 mM NaCl. As membranes are an alternative to resin-packed columns in GMP productions, it was important to demonstrate that this method is effective in evaluating such systems.

Given the previously established relationship between MVM-VLP and MVM clearance results,[18–21] the data generated by the presented platform are useful for quickly determining viral clearance feasibility of various chromatography methods. The methods presented herein are not intended to supplant traditional viral clearance validation studies; however, this high-throughput surrogate-based technique enables in-house examination of the process parameters and unique test articles during early development. Providing process-specific viral clearance knowledge rather than relying on historical data, theoretical models, or published comparisons, successfully de-risks a critical process development goal.

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CONFLICT OF INTEREST

David Cetlin is a salaried employee of Cygnus Technologies, LLC the developer of the MockV MVM Kit.

AUTHOR CONTRIBUTIONS

CASRAI CRediT Taxonomy: authors' contribution(s) to the submitted manuscript are attributed as follows: CRediT Taxonomy. K.G.: Conceptualization (Equal); Data curation (Equal); Formal analysis (Equal); Methodology-Equal, Supervision (Equal); Writing-original draft (Lead). Zachary Schneiderman: Conceptualization (Equal); Data curation (Equal); Formal analysis-(Equal); Investigation (Equal), Writing-original draft (Equal); Sarah O'Connell: Data curation-Equal, Formal

analysis-Equal, Methodology (Equal); Writing-review & editing (Equal). G.A.: Investigation (Supporting); Writing-review & editing (Equal). N.C.: Data curation (Equal), Visualization-Lead, Writing-review & editing-Equal. D.C.: Conceptualization-Equal, Resources (Equal); writing-review & editing (Equal). D.G.: Conceptualization (Equal); Data curation-Equal, Project administration (Lead), Supervision (Equal), Writing-review & editing (Equal).

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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