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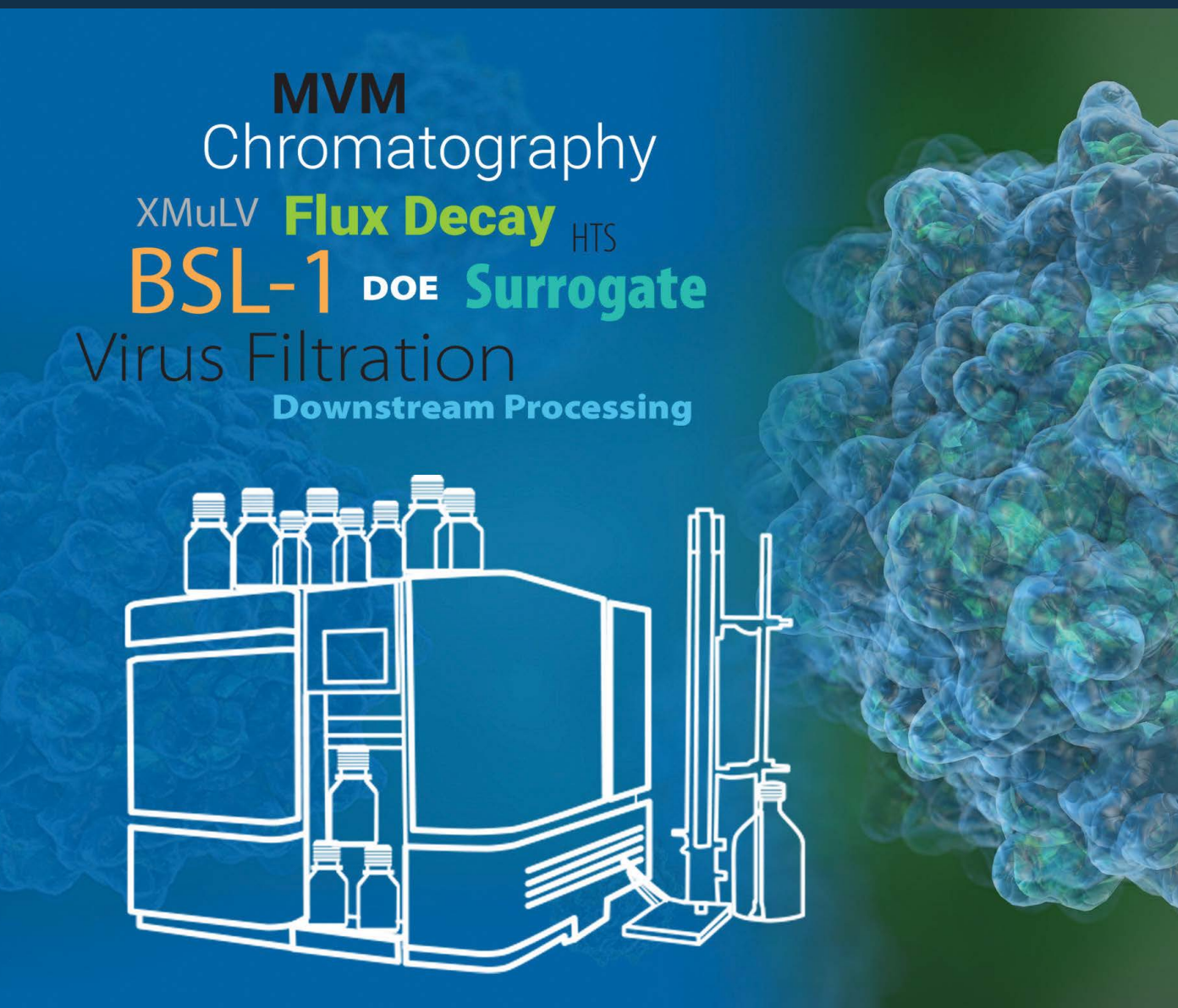
Predicting Viral Clearance in Downstream Process Development



Predicting Viral Clearance in Downstream Process Development

Application of a Novel Analytical Approach

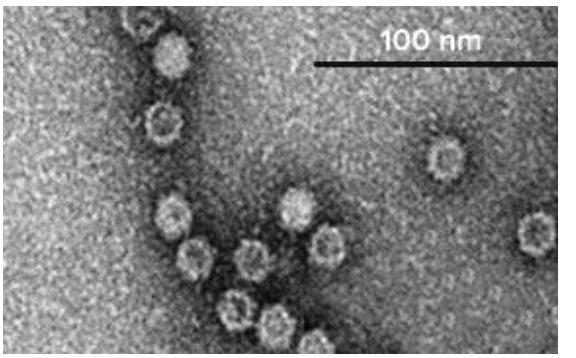
David Cetlin

Biopharmaceuticals are the products of complex manufacturing processes. They are approved for clinical and (later) commercial use only after their manufacturing processes meet specific safety and quality standards. Each process requires techniques that purify finished drug product from initial cell-culture solution. The science of selecting and optimizing such techniques is called *downstream process development*. Its major focus is to assess a process's ability to remove impurities that originate from source materials, including host DNA and host-cell proteins (HCPs). Commercially available, low-cost, assay kits are used routinely to quantify impurities, guiding scientists throughout process development toward the establishment of a robust and scalable manufacturing process. However, until recently, similar kits for viral-clearance assessments have been unavailable.

Viral Contamination

Viral contamination is an inherent risk during biopharmaceutical manufacturing. Whether introduced endogenously from cell banks or exogenously during manufacturing, unmitigated viral contaminations have led to serious health implications, including transmission of influenza, human immunodeficiency virus (HIV), hepatitis, herpes, measles, and poliomyelitis (**1**). International regulatory agencies, therefore, require biopharmaceutical companies to validate the viral clearance efficacy of their manufacturing processes before clinical trials and commercial approvals.

Currently, validation is performed through small-scale *spiking studies*, whereby specific model mammalian viruses (e.g., minute virus of mice, MVM) are introduced artificially into biopharmaceutical material and cleared (removed or inactivated) by a subsequent purification technique. Spiking studies require specialized biological safety level (BSL) laboratories and sufficiently trained personnel, resulting in costs that can soar over $US100,000.



Transmission electron microscopy (TEM) image of noninfectious mock virus particles (MVPs)

Such hurdles deter companies from analyzing viral clearance during small-scale process development. Instead, sponsors delay assessments and spend considerable resources up front optimizing purification process steps without knowing their viral-clearance efficacy. However, that increases the risk of validation failure and can force companies to invest additional time and money redeveloping process steps when failures occur.

A Practical Solution

One solution to the viral-clearance dilemma is an economical, practical, and accurate way to assess clearance efficacy as a routine experimental output. In 2020, Cygnus Technologies introduced the MockV MVM assay kit to address the biopharmaceutical industry's needs at the intersection of viral clearance and process development. This offers a first-of-its-kind, ready-to-use solution — the first in a series of upcoming kits that will enable scientists to predict viral clearance in house within a BSL-1 setting.

Each kit contains a noninfectious viral surrogate, a *mock virus particle* (MVP), that mimics the physicochemical properties of a model live virus typically used in clearance validation studies. Strong resemblance between MVPs and live virions enables accurate viral clearance prediction across different

Figure 1: (A) Schematic of an immunoquantitative polymerase chain reaction (immuno-qPCR) assay; (B) sample standard curve data from that assay (**5**); MVM = minute virus of mice, MVP = mock virus particle

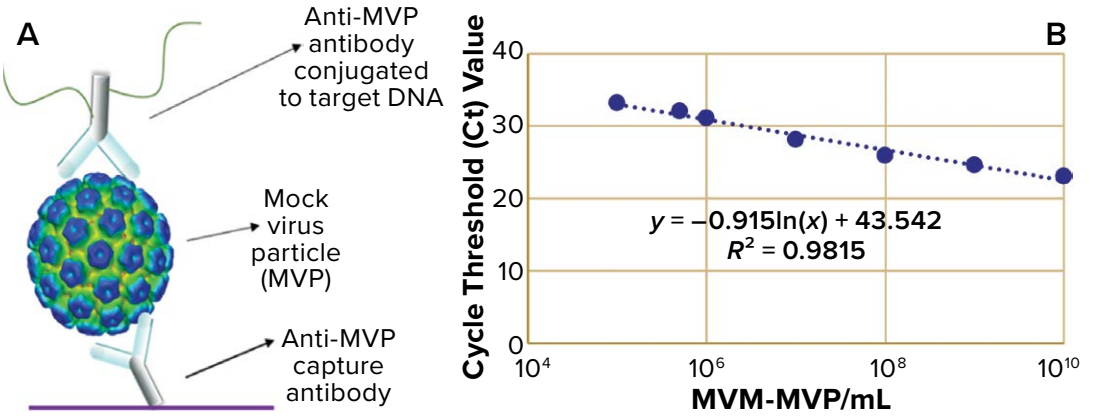


Figure 2: Absorbance profiles of MVPs eluting from monoclonal-antibody (MAb)–conjugated resin (**3**)

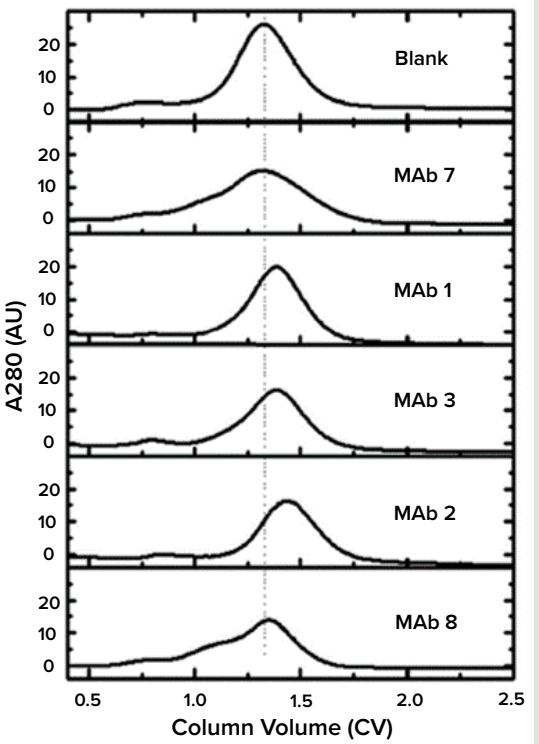


Table 1: Results from physicochemical comparison of minute virus of mice (MVM) and mock virus particles (MVPs); hydrodynamic radii and diameter measurements were obtained by multiangle light scattering (MALS) and transmission electron microscopy (TEM), respectively. Hydrophobicity values listed in the table indicate relative hydrophobic affinity to phenyl (1.0 = insulin).

Parameter	Live MVM	MVM-MVPs
Hydrodynamic radii	18.4 ± 0.2 nm	17.2 ± 0.1 nm
Diameter	24.6 ± 3.6 nm	25.6 ± 3.0 nm
Surface charge (pI)	5.99	5.81
Hydrophobicity	0.28	0.35

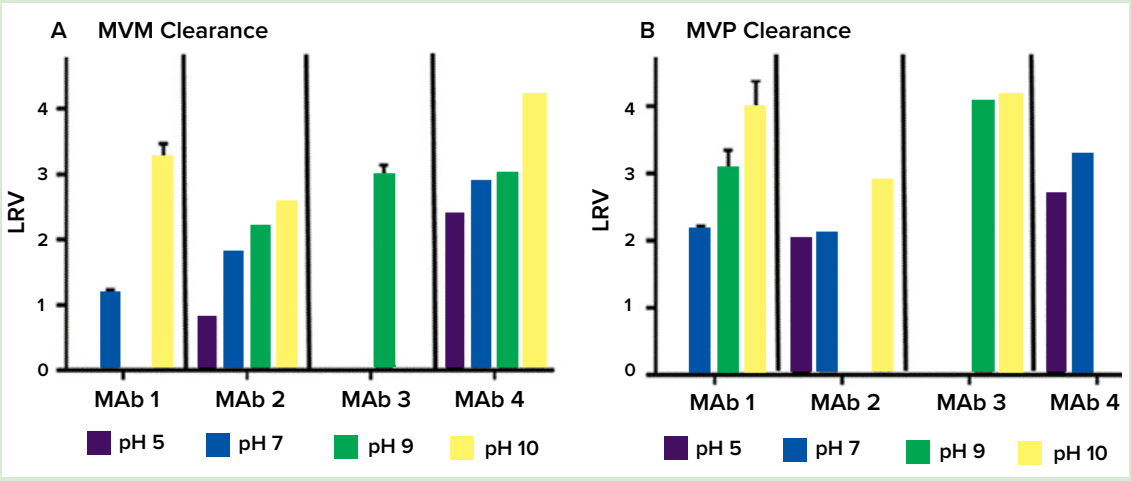
Table 2: Impact of different feedstreams on log reduction values (LRVs) during protein A clearance of mock virus particles (MVPs); PBS = phosphate-buffered saline, MAb = monoclonal antibody

	Elution Pool LRV	
Feed Stream	pH 9 Wash	pH 10 Wash
PBS	≥ 4.21	≥ 4.46
Null cell harvest	≥ 4.01	≥ 4.39
Purified MAb	3.11	2.64
Null cell harvest + purified MAb	3.21	≥ 4.65

modes of chromatography and nanofiltration. Thus, the MockV MVM kit offers process development scientists a novel tool for generating viral-clearance data conveniently throughout a biopharmaceutical's lifecycle, with applications spanning high-throughput screening, process development, process characterization, and manufacturing-deviation support. Using MVP kits to support a quality-by-design (QbD) approach, scientists can optimize purification steps and determine with confidence whether process steps and parameters are effective before investing significant resources in regulatory-supporting validation studies using live virus.

The commercially available MockV MVM kit contains a BSL-1 surrogate that mimics the physicochemical properties of MVM, a parvovirus that is used internationally as a model virus for spiking studies. These "MVM-MVPs" are produced by

Figure 3: Impact of protein A wash 2 buffer pH on log reduction values (LRVs) for clearance of minute virus of mice (MVM) (A) and mock virus particles (MVPs) (B) (**3**)



expressing MVM's major capsid protein, VP2, in a recombinant system and then purifying the assembled particles.

A 2017 collaboration with the US Food and Drug Administration (FDA) studied the physical and chemical properties of MVM-MVPs alongside those of live MVM (**2**). Results from the agency's experiments demonstrated the similarity of the particles' size, surface-charge, and surface-hydrophobicity profiles (Table 1). A 2020 study published by researchers from Bristol Myers Squibb (BMS) confirmed that strong physicochemical resemblance (**3**).

To use MVM-MVPs, load material is spiked with the surrogate, then processed through the desired separation technique. Collected fractions are analyzed for MVP concentration, and log reduction values (LRVs) are calculated. To quantify MVP concentration in solution, Cygnus Technologies has developed an immuno-quantitative polymerase chain reaction (immuno-qPCR) assay. (Figure 1A). The highly sensitive technique enables quantification of MVP concentrations over a 4.0–5.0 $\log_{10}$ dynamic range, allowing for LRV determinations between 4.0 and 5.0 (Figure 1B). Each MockV MVM kit contains ample immuno-qPCR reagents and MVP to conduct ~10 small-scale experiments, assuming a spiked load concentration of 10^9 particles/mL and a volume of 150 mL.

Case Studies of MVM-MVP Utility

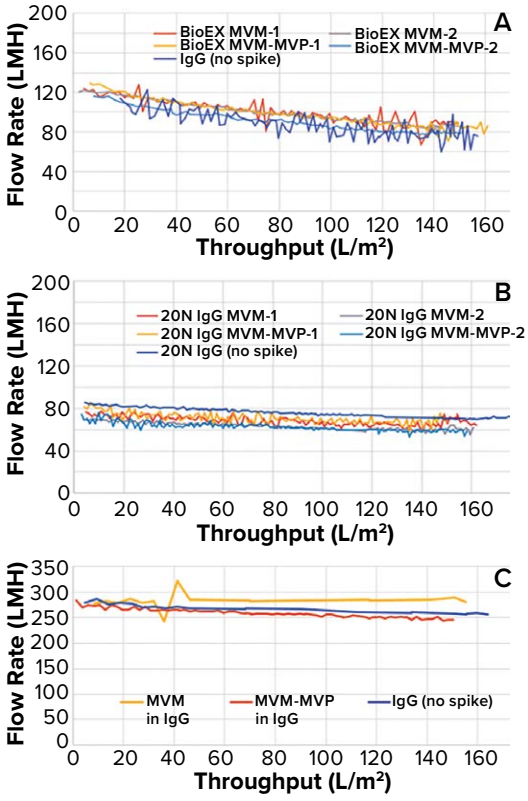
Several published studies have demonstrated the comparable clearance of MVM-MVPs and live MVMs over a wide range of monoclonal antibody (MAb) downstream applications, including protein A affinity chromatography (**3**), nanofiltration (**4–6**), anion-exchange chromatography (AEX) (**6, 7**), and hydrophobic-interaction chromatography (HIC) (**8**). Data also have been gathered from gene therapy process-development workflows (**9**) and used to provide predictive viral clearance outputs for design of experiments (DoE) (**7**) and high-throughput screening (HTS) studies (**8**).

Viral Clearance During Protein A Chromatography: In 2020, BMS researchers used MVP surrogates to study the mechanistic behavior of MVM clearance during protein A chromatography (**3**). To examine potential interactions between MVM-MVPs and MAbs, several MAbs were conjugated to the base matrix of a Sepharose resin (Cytiva) and packed into separate columns. Each column was spiked with 100 µL of MVPs from a MockV MVM kit. Then, the BMS team ran the columns and measured the particles' retention time by tracking their UV absorbance at 280 nm.

Results from those spiking studies revealed different absorbance profiles for each MAb, suggesting that interactions with MVPs differ among antibodies (Figure 2). MAb-specific interaction might contribute to varying LRVs across MAb processes, even if operated under the same chromatographic conditions (e.g., using a platform process). These findings align with the established variability of MVM clearance in protein A capture steps (**10–12**).

Next, BMS scientists studied the impact of protein A wash pH on MVP and MVM clearance. They established that MVM LRV improvements could be gained by increasing the wash 2 pH and that MockV MVM kits enabled accurate prediction of such changes in LRVs during those studies (Figure 3).

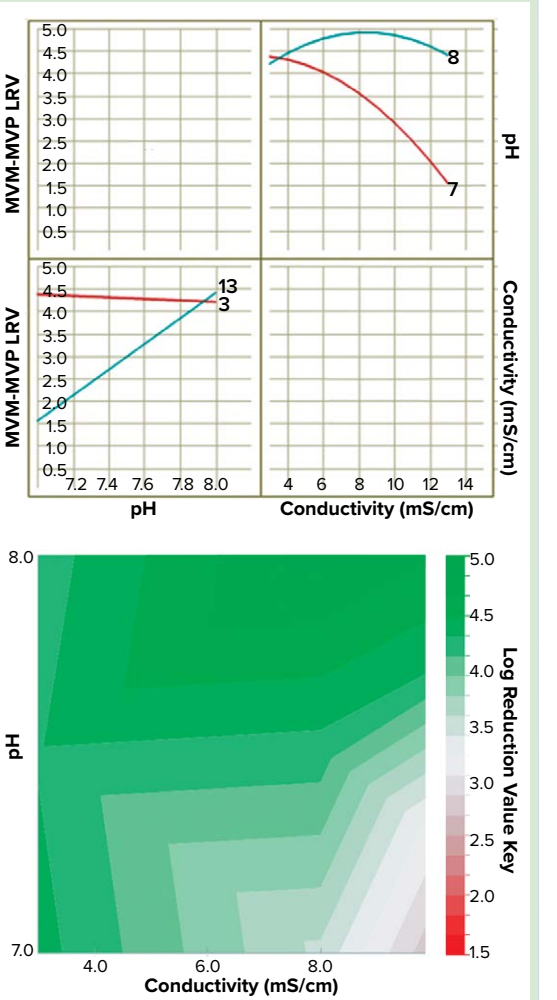
Figure 4: Flux-decay profiles for nanofiltration of minute virus of mice (MVM), mock virus particles (MVPs), and IgG using Planova BioEX (A), 20N (B), and 35N (C) filters; the table (BOTTOM) shows log reduction values (LRVs) from n = 2 MVM and MVP experiments for each filter type (ND = not determined).



	MVM 1	MVM 2	MVP 1	MVP 2
BioEX	≥4.7	≥4.8	≥3.5*	≥3.6*
20N	≥4.8	≥4.8	≥3.2*	≥2.8*
35N	0.1	ND	0.4	ND

* Results from a first-generation immuno-qPCR assay (higher limit of detection)

Figure 5: Interaction plot (TOP) and response surface graph (BOTTOM) illustrating the effects of pH and conductivity on mock virus particle (MVP) clearance (**7**)



The BMS team also spiked MVPs into different MAb feedstreams to assess the impact of feedstreams and associated impurities (e.g., HCPs and DNA) on MVP clearance during protein A separation. Complete MVP clearance was achieved from phosphate-buffered saline (PBS) and null cell-harvest material lacking MAbs (with LRVs ≥ 4.2/4.46 and ≥ 4.01/4.39, respectively) (Table 2). Those results indicate that without MAbs present, MVPs associate loosely with protein A and thus will wash away before elution. MVP removal diminished when using a purified MAb feedstream (3.11/2.64), indicating that the particles interacted with the product and may have "piggy-backed" their way into an elution pool. LRVs also were low (3.21) when impurities were added to the purified MAb feedstream and a wash pH of 9.0 was applied. However, full clearance was restored (≥4.65) by increasing wash buffer pH to 10, which effectively reduced the amount of impurities.

Those data suggest that MVP–MAb interaction plays a role in MVP removal but that clearance ultimately is dictated by MVP interactions with impurities. Overall, the BMS study exemplifies the type of development work that can be performed using the MockV MVM kit.

Nanofiltration Applications: To demonstrate the MockV MVM kit's ability to predict MVM removal during nanofiltration processes, IgG-containing solutions were spiked (in parallel) with live MVMs (Texcell) or MVM-MVPs, then processed through Planova 20N, BioEX, or 35N nanofiltration devices (Asahi Kasei). Flux-decay data were collected, and particle reduction values were calculated based on

Figure 6: Clearance of live MVM and MVPs using POROS benzyl hydrophobic-interaction chromatography resin (blue, bind–elute mode) and POROS Benzyl Ultra resin (orange, flow-through mode); BSL = biosafety level

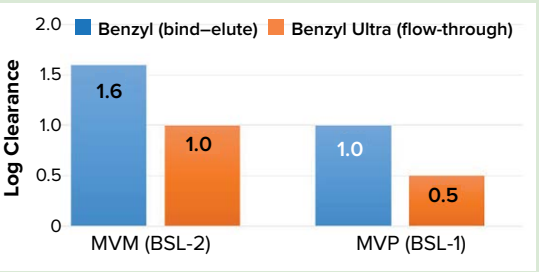


Figure 7: Log reduction values (LRVs) from POROS AAVX runs spiked with either MVM or MVPs (**9**)

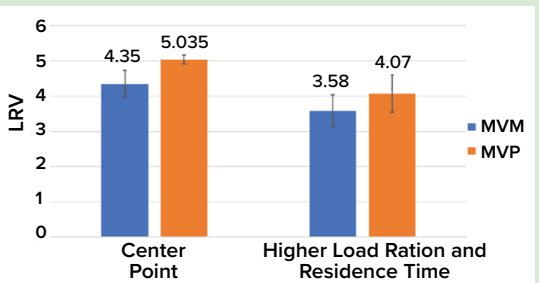
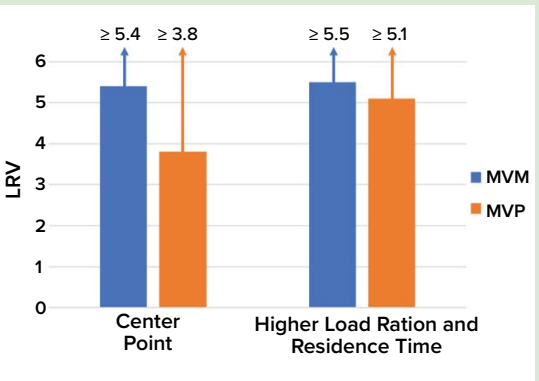


Figure 8: Log reduction values (LRVs) from CIMmultus QA runs spiked with either MVM or MVPs (**9**)



median tissue culture infectious dose ($TCID_{50}$) and immuno-qPCR analyses. As shown in Figure 4, the mock- and live-virion processes exhibited comparable filtration performance and particle reduction (**4**).

Early in 2021, researchers from Gakushuin University in Japan published about their use of fluorescently labeled MVPs to monitor nanofiltration dynamics in real time through ultrastable optical microscopy (**5**). Facilitated by a noninfectious MVM surrogate, the group's method yielded new insights into the mechanisms of virus filtration through fiber membranes.

Table 3: Mass balance data from POROS AAVX runs spiked with either live MVM or noninfectious MVPs at center-point (CP) or worst-case (WC) conditions (**9**)

		Total Particles (log_{10})			Percentage of Particles		
			MVM-MVP			MVM-MVP	
Run	Phase	MVM	Run 1	Run 2	MVM	Run 1	Run 2
CP	Load	8.1	12.3	12.2	NA	NA	NA
	FT	7.9	12.0	12.0	66.1%	52.6%	67.0%
	Wash 1	6.1	10.0	9.7	1.0%	0.5%	0.3%
	Wash 2	5.4	11.3	11.2	0.2%	10.4%	10.4%
	Wash 3	4.7	8.7	8.7	0.0%	0.0%	0.0%
	Elution	3.8	7.4	7.0	0.0%	0.0%	0.0%
	CIP	5.0	6.9	6.7	0.1%	0.0%	0.0%
WC	Load	7.9	11.9	NT	NA	NA	NA
	FT	7.6	11.8	NT	55.0%	79.1%	NA
	Wash 1	NT	9.9	NT	NA	1.1%	NA
	Wash 2	NT	11.0	NT	NA	14.3%	NA
	Wash 3	NT	9.0	NT	NA	0.1%	NA
	Elution	4.3	7.8	NT	0.0%	0.0%	NA
	CIP	NT	6.8	NT	NA	0.0%	NA

NT = not tested; **NA** = not applicable; **FT** = flow-through fraction; **CIP** = clean-in-place solution

DoE Assessment of an AEX Polishing Step: Scientists at AstraZeneca (**6**) and GlaxoSmithKline (**7**) have demonstrated the utility of the MockV MVM kit for AEX purification of MAbs. In the latter study, researchers conducted a full-factorial, central composite face DoE examining load pH and conductivity. LRVs generated through 10 MVP-spiked runs were entered into JMP software, and a statistically significant/valid model was built (R^2 = 0.92, $p < 0.01$, no lack of fit). A two-dimensional response-surface graph and an interaction plot were constructed to depict the general trend of MVP clearance and expected LRV outcomes within the tested operational space (Figure 5). Those results confirmed that a MockV MVM kit could predict the influences of process parameters on LRV quickly and cost-effectively.

HIC and Viral Clearance: Compared with other forms of chromatography, the ability of HIC resins to remove viruses has garnered little attention. A 2019 study by Thermo Fisher Scientific showed minimal to modest clearance of MVM using POROS HIC resins (**8**). To investigate the role of hydrophobicity in MVM binding, the company used a MockV MVM kit as an economic MVM surrogate in a HTS format. Binding of a MAb load spiked with MVPs was tested in POROS benzyl, Benzyl Ultra, and ethyl resins under increasing concentrations of sodium citrate. The

Table 4: Mock virus particle (MVP) clearance as represented by log reduction values (LRVs) gathered during high-throughput screening of anion-exchange chromatography (AEX) resins

AEX Resin Screen	Load FT	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl	250 mM NaCl	300 mM NaCl	500 mM NaCl	1 M NaCl
Resin 1, pH 6.5	4.8	4.66	4.55	0.75	0.69	0.67	0.67	0.66	0.66
Resin 1, pH 7.5	4.8	4.66	4.55	3.18	0.94	0.86	0.84	0.82	0.82
Resin 1, pH 8.5	4.8	4.66	4.55	1.19	0.94	0.87	0.87	0.86	0.86
Resin 2, pH 6.5	4.8	4.66	4.55	4.47	4.4	4.34	4.28	0.98	0.93
Resin 2, pH 7.5	4.8	4.66	4.55	4.47	4.4	4.34	4.28	0.73	0.71
Resin 2, pH 8.5	4.8	4.66	4.55	4.47	4.03	3.81	3.67	0.85	0.83
Resin 3, pH 6.5	4.8	4.66	4.55	4.06	0.66	0.65	0.65	0.65	0.65
Resin 3, pH 7.5	4.8	4.12	4.09	4.06	2.08	0.95	0.94	0.93	0.93
Resin 3, pH 8.5	4.8	4.66	4.55	4.47	4.4	0.97	0.95	0.94	0.94
Resin 4, pH 6.5	4.8	4.66	1.4	0.64	0.63	0.63	0.63	0.63	0.63
Resin 4, pH 7.5	4.8	4.66	4.55	1.16	0.73	0.72	0.72	0.72	0.72
Resin 4, pH 8.5	4.8	4.66	4.55	2.01	0.77	0.74	0.73	0.73	0.73
Resin 5, pH 6.5	4.8	4.66	4.55	4.47	2.45	1.08	0.91	0.89	0.89
Resin 5, pH 7.5	4.8	4.66	4.55	4.47	4.4	2.3	1.0	0.74	0.73
Resin 5, pH 8.5	4.8	4.66	4.55	4.06	4.03	3.81	1.37	0.84	0.83
Resin 6, pH 6.5	4.8	4.66	4.55	0.61	0.54	0.53	0.53	0.53	0.53
Resin 6, pH 7.5	4.8	4.66	4.55	4.47	0.57	0.49	0.48	0.48	0.48
Resin 6, pH 8.5	4.8	4.66	4.55	4.47	1.14	0.81	0.8	0.8	0.79
Resin 7, pH 6.5	4.8	4.66	4.55	1.87	0.79	0.77	0.77	0.77	0.73
Resin 7, pH 7.5	4.8	4.66	4.55	4.47	0.98	0.58	0.57	0.56	0.56
Resin 7, pH 8.5	4.8	4.66	4.55	1.09	0.79	0.78	0.77	0.77	0.77

resins all showed low MVP retention even at the highest salt concentration (600 mM of sodium citrate), indicating the low overall hydrophobicity of MVPs. Those findings correlate with the minimal clearance of MVM observed in processes using HIC resins.

Next, parallel MVM and MVP spiking studies were performed with POROS benzyl resin in bind–elute mode and with POROS Benzyl Ultra resin in flow-through mode. Results from those head-to-head studies demonstrated the high LRV correlation between the two particle types and confirmed the MockV MVM kit's ability to predict MVM clearance (Figure 6).

Gene Therapy Process Development: Viral clearance is important not only to MAb operations, but also to purification of gene therapies. A particular challenge for such processes is that the target (e.g., adenoassociated virus, AAV) is essentially a virus or virus-associated particle. That renders useless some of the separation techniques that commonly are used for MAbs (e.g., nanofiltration). To that end, researchers from Regenxbio studied the efficacy of affinity and AEX separations for viral clearance using POROS CaptureSelect AAVX resin (Thermo Fisher) and CIMmultus QA monoliths (BIA Separations, a Sartorius company), respectively (**9**). During that study, MockV MVM kits were used in parallel to MVM spiking experiments.

Data from the AAVX resin centerpoint and worst-case operating parameters showed high correlations between MVM and MVP mass balances throughout the runs (Table 3). Overall, elution LRVs for both particle types correlated strongly, exhibiting the same ~1 $\log_{10}$ decreases in LRV during worst-case conditions (Figure 7). Likewise, data from CIMmultus QA monolith experiments showed a high correlation between MVP and MVM mass balances under both centerpoint and worst-case conditions (Figure 8). Overall, Regenxbio's experiments demonstrated the high predictive value of MockV MVM kits for estimation of MVM clearance during AAV processes.

High-Throughput Resin Screening: Cygnus Technologies collaborated with the Vaccine Research Center of the National Institutes of Health to perform high-throughput evaluation of several AEX resins' viral-clearance efficacy. The resins were screened across a wide range of pH and conductivity

The resins differed greatly in their retention of MVPs as salt concentrations increased. Some resins retained them after exposure to moderate and high salt concentrations, whereas others did not. These studies exemplify how large amounts of viral-clearance–prediction data can be collected QUICKLY and ECONOMICALLY using a MockV MVM kit.

conditions. Robocolumns from multiple vendors were equilibrated with buffers containing 10 mM NaCl (pH 6.5, 7.5, and 8.5). Then, pH-adjusted loads containing a vaccine candidate were spiked to 1×10^{11} MVP/mL and added to each column. The plate containing the columns was mixed and centrifuged while flow-through was collected. A series of buffers with increasing NaCl concentrations was added to the columns. After each addition, the plate was mixed and centrifuged, and flow-through was collected. All collected samples were analyzed for MVPs, and LRVs were calculated.

The resins differed greatly in their retention of MVPs as salt concentrations increased (Table 4). Some resins (e.g., 3 and 8) retained them after exposure to moderate and high salt concentrations, whereas others (e.g., 1) did not. These studies exemplify how large amounts of viral-clearance–prediction data can be collected quickly and economically using a MockV MVM kit.

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