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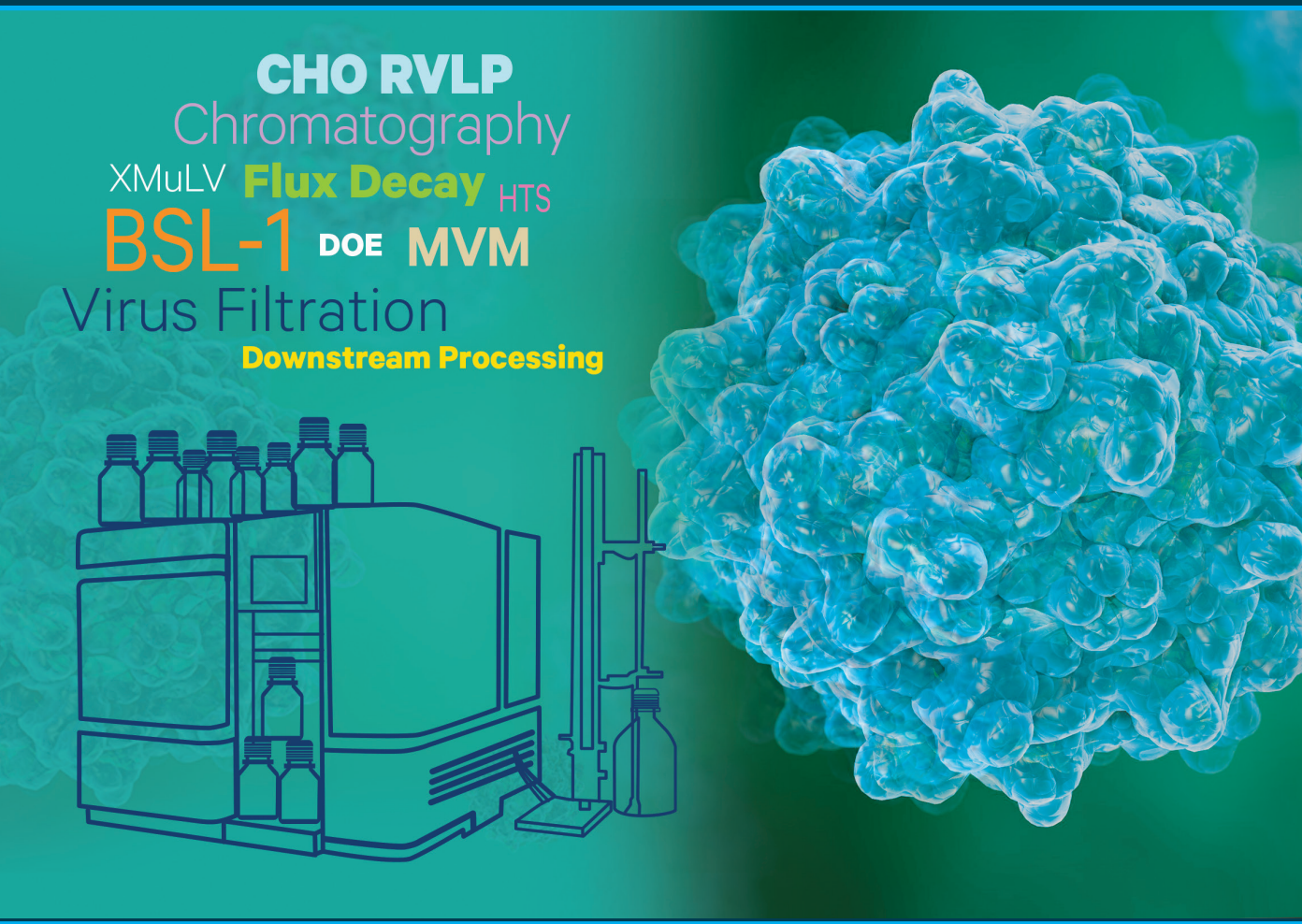
SPECIAL REPORT

Viral Clearance in the Age of Q5A(R2) and the Case for Using Noninfectious Spiking Agents



Viral Clearance in the Age of Q5A(R2) and the Case for Using Noninfectious Spiking Agents

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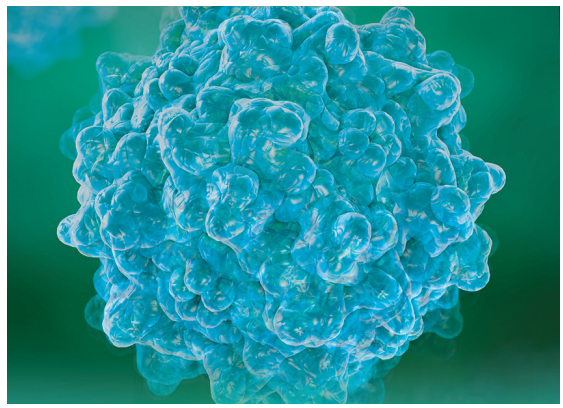
Viral contamination is an inherent risk during biopharmaceutical manufacturing. Whether introduced endogenously from cell banks or exogenously during processing, unmitigated viral contaminations have occurred, albeit infrequently, in the past (**1**). Such events can cause serious health implications (**2**). Thus, international regulatory agencies require biopharmaceutical companies to validate the viral-clearance efficacy of their manufacturing processes prior to granting approval for clinical trials or commercial release (**3**). Currently, those studies are performed using small-scale "spiking studies," in which specific model mammalian viruses — e.g., minute virus of mice (MVM) and xenotropic murine leukemia virus (XMuLV) — are introduced artificially ("spiked") into biopharmaceutical material and then cleared (removed or inactivated) by a purification technique.

Spiking studies require laboratories equipped for high biological safety levels (BSLs) and specially trained personnel. Most drug developers perform these studies through contract research organizations (CROs), which can result in logistical difficulties, lengthy timelines (for both study execution and data reporting), and costs that can soar well above US$100,000. Such hurdles deter many developers from studying their viral-clearance capabilities during small-scale process development and optimization. Instead, they delay assessments and spend considerable upfront resources optimizing purification process steps without knowledge of their viral-clearance efficacy. That strategy increases the risk of validation failure, which forces companies to invest additional time and resources into redeveloping process steps.

Viral-Safety Evaluation of Animal-Cell–Derived Biotechnology Products

In September 2022, global regulatory agencies, including the US Food and Drug Administration

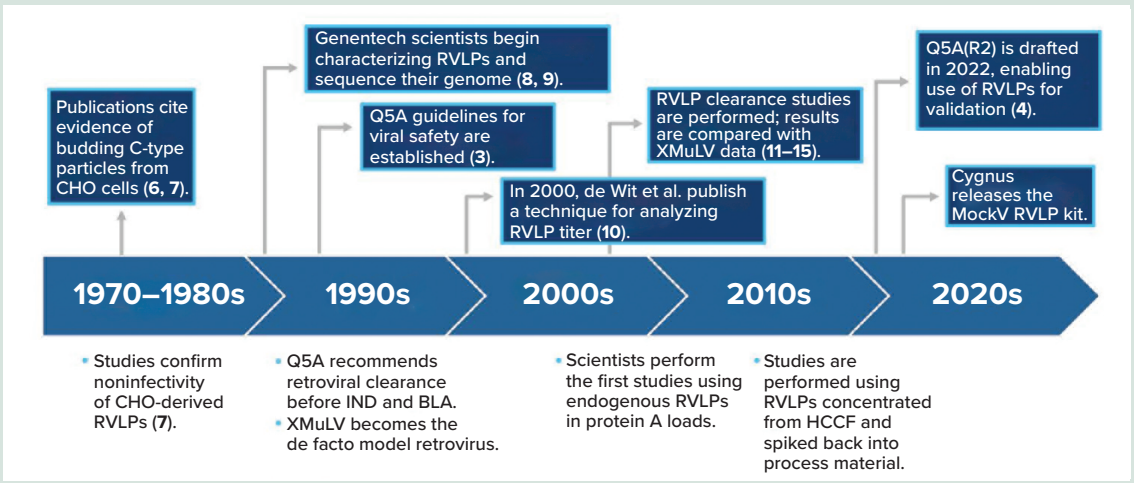


(FDA) as part of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), drafted a second revision (R2) to the Q5A guidance that governs the biotechnology industry on the topics of viral safety and its evaluation (**4**). The draft Q5A(R2) document provides an update to the regulatory stance on viral safety and addresses many of the technological advancements that have occurred since the enactment of the first revision (R1) in 1997, including viral-clearance considerations for continuous processing, cell/gene therapy products, and application of prior knowledge. When used appropriately, prior knowledge can help to reduce the dependence and scope of CRO-led viral-clearance validation studies for a specific biopharmaceutical product. *Prior knowledge* consists of

- in-house knowledge gained as a biomanufacturer uses established platform process steps to purify similar and well-characterized products
- external knowledge in the form of publicly available published data
- established scientific principles.

Annex 6 of Q5A(R2) provides an example of how a company could leverage prior knowledge to limit

Figure 1: Advances in the study of retrovirus-like particles (RVLPs) endogenous to Chinese hamster ovary (CHO) cells



the scope of a spiking study for viral-clearance validation. The example makes clear that successful application of prior knowledge depends on a solid justification, including "a discussion of all the data available and the rationale to support the approach" (**4**). As Perez-Caballero et al. summarize in their review of Q5A(R2), prior-knowledge data should help

- provide a good understanding of the mechanism underlying virus clearance
- account for all process parameters affecting viral clearance
- demonstrate the influences of interactions between viruses and biological products
- consider potential for viral-clearance interference based on the composition of some process intermediate(s) (**5**).

The bar is set high for implementing prior knowledge, and a drug developer must provide considerable data to justify doing so. Through its MockV product line, Cygnus Technologies offers solutions that ease the accumulation of viral-clearance data. Using these solutions, companies can increase process knowledge, helping to support justifications. Supplementing internal prior knowledge with viral-clearance data from MockV technology could reduce the need for — and the scope of — process-specific live-viral validation spiking studies.

The MockV Approach

Above, we noted the high costs, logistical complexity, and lengthy timelines that come with gaining viral-clearance data through a CRO. Drug developers need an economical, practical, and accurate way to assess viral-clearance efficacy using a routine experimental output. In 2020, Cygnus Technologies introduced the MockV product line to address industry needs at the intersection of viral clearance and process development. The first of their kind, MockV ready-to-use kits enable scientists to track viral clearance in house within a BSL-1 setting. Each kit contains specific noninfectious viral particles that can be spiked into downstream bioprocess material and then quantified after chromatography or nanofiltration.

The kits provide scientists with novel means for conveniently generating viral-clearance data throughout a biopharmaceutical's life cycle, with applications possible for high-throughput screening, process development, process characterization, process validation, and manufacturing-deviation support. Leveraging the kits and a quality by design (QbD) approach, scientists can confidently optimize purification steps for virus removal and determine whether process steps and parameters are effective before investing significant resources in regulatory-supporting spiking studies for viral-clearance validation. As noted above, the MockV approach also can be used to enhance prior knowledge, helping to build a case for limiting live-virus validation studies.

Retrovirus-Like Particles (RVLPs)

In November 2022, Cygnus Technologies launched the MockV RVLP kit. Each kit contains a highly concentrated and purified stock solution of noninfectious RVLPs derived from Chinese hamster ovary (CHO) cells. The particles can serve as a BSL-1–compatible spiking agent for viral-clearance testing. Potential applications go beyond process development.

As the timeline in Figure 1 illustrates, early evidence of budding C-type particles from CHO cells

Figure 2: (A) Transmission electron microscopy (TEM) image of Chinese hamster ovary (CHO) cell retrovirus-like particles (RVLPs); dynamic light scattering analysis of (B) CHO-RVLPs and (C) xenotropic murine leukemia virus (XMuLV)

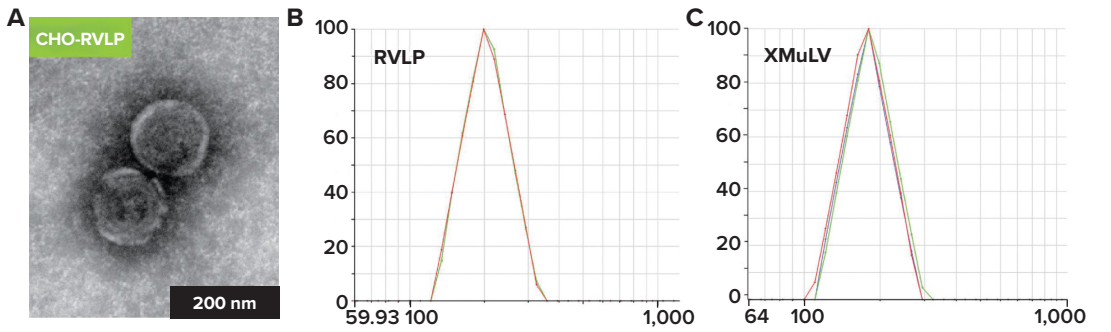


Table 1: Data from dynamic light scattering analysis of CHO-RVLPs and XMuLV; Eff. Dia. = efficient diameter (in nanometers), PD = polydispersity

Sample	Eff. Dia.	PD	Sample	Eff. Dia.	PD
RVLP-1	194 nm	0.10	XMuLV-1	243 nm	0.12
RVLP-2	193 nm	0.09	XMuLV-2	0.12 nm	0.10
RVLP-3	193 nm	0.09	XMuLV-3	242 nm	0.11
Mean	193 nm	0.09	Mean	0.10 nm	0.11
Std. error	0.4	0.00	Std. error	242	0.01
Std. dev.	0.8	0.01	Std. dev.	0.11	0.01

The ICH Q5A guidance states, "For CHO cell-derived products, CHO-derived ENDOGENOUS VIRUS PARTICLES can also be used for viral clearance experiments."

was documented in the 1970s and 1980s (**6, 7**) (Figure 1). In the early 1990s, scientists from Genentech/Roche began to characterize the production of those particles (**8**). Although RVLPs have proven to be noninfectious (**7**), their discovery prompted regulators to require demonstration of retrovirus clearance before clinical trials or market approval (**3**). To accomplish that, the biopharmaceutical industry adopted XMuLV as a model retrovirus for CRO-led spiking studies. In the meantime, scientists continued to characterize CHO-endogenous RVLPs, sequencing their proviral genomes (**9**) and using that information to develop highly sensitive detection assays based on reverse-transcription quantitative polymerase chain reaction (RT-qPCR) (**10**). Then, process development scientists could leverage such advances to conduct clearance studies with CHO-endogenous RVLPs.

The initial studies used RVLPs present in harvest cell-culture fluid (HCCF) to determine viral-clearance capability during protein A separation, which is typically the first process step in purifying monoclonal antibodies (MAbs) derived from CHO cells (**11, 12**). Those early studies demonstrated not only the feasibility of RVLP-based determination of viral clearance, but also the comparability of RVLP clearance with XMuLV clearance, which had been the gold-standard method for measuring retroviral clearance during downstream processing of biologics. Later studies used RVLPs to examine the contributions of further downstream processes to clearance, namely anion-exchange (AEX) chromatography steps (**13–15**). To accomplish that testing, RVLPs were spiked back into process samples because most endogenous particles had been depleted during protein A separation and/or viral inactivation. Those studies further highlighted the utility of RVLPs for demonstrating retroviral clearance.

As we highlight above, global regulatory agencies recently drafted a second revision to the Q5A guidance that governs the biotechnology industry on viral safety and its evaluation. The document states, "For CHO cell-derived products, CHO-derived endogenous virus particles can also be used for viral clearance experiments" (**4**). Thus, RVLPs can be used to validate the retroviral-clearance efficacy of downstream process steps.

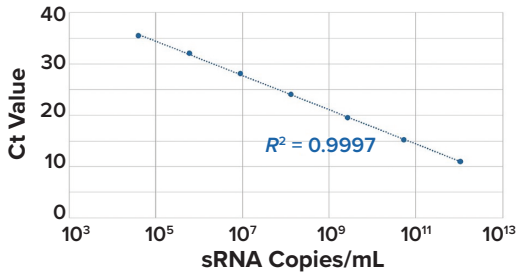
The MockV RVLP Kit

The Cygnus Technologies MockV RVLP kit is a complete, ready-to-use product that enables purification scientists to apply RVLPs for downstream spiking applications beyond process development. The MockV approach overcomes the

RVLP Stock Solution

Each Cygnus MockV RVLP kit contains a stock solution of noninfectious RVLPs. The particles are produced during Chinese hamster ovary (CHO) cell cultivation and purified through multiple modes of chromatography, enriching and concentrating the RVLPs to 10^{10}/mL. The solution is qualified using dynamic light scattering (DLS), which calculates the particles' polydispersity and size distribution (Table 1). Characterization data for the stock solution also include transmission electron microscopy (TEM) images, which help users determine particle morphology and perform comparative DLS against XMuLV samples (Figure 2).

Figure 3: RNA standard (sRNA) curve for the RVLP reverse-transcription quantitative polymerase chain reaction (RT-qPCR) assay; Ct = cycle threshold



cost and complexity barriers of live-XMuLV studies because RVLPs can be handled safely on a laboratory benchtop.

Each kit includes a vial of CHO-derived RVLP stock solution (see the "Stock Solution" box, Figure 2, and Table 1), a 96-well plate for sample analysis, reagents for RNA extraction and qPCR, and a well-controlled RNA standard for accurate and reliable RVLP quantification. By following the kit's straightforward protocol, scientists can detect as little as 10^3 RVLP/mL of sample, enabling log reduction values (LRVs) of ~5.0 to be determined. Each kit contains 2.0 mL of RVLP stock solution at a concentration of 10^{10} RVLP/mL, which is sufficient for spiking up to 200 mL of load material to 1% (v/v) and conducting analysis on 23 samples in triplicate in less than one day. A real-time qPCR instrument is required along with standard laboratory equipment, and kit users need minimal experience with RNA extraction and qPCR protocols. Compatible purification steps include virus filtration and AEX, cation-exchange (CEX), hydrophobic-interaction (HIC), mixed-mode, protein A, and size-exclusion (SEC) chromatographies.

Analytical Assay and Qualification: To analyze the concentration of RVLPs in samples, the MockV RVLP kit provides components for RNA extraction and RT-qPCR. Samples are added to a 96–deep-well plate and treated with an endonuclease to degrade proviral RVLP DNA sequences or exogenous RVLP RNA. Then, RNA is extracted and precipitated with a set of proprietary buffers. The plate is stored at –20 °C for 30 minutes, and the RNA is pelleted by centrifugation at 3,000*g* for 20 minutes at 4 °C. After washing and final pelleting, the RNA is resuspended in a proprietary buffer, and 10 µL of sample is transferred from each well of the plate to a qPCR plate containing TaqMan primers/probes (Thermo Fisher Scientific) directed against the DNA polymerase (*pol*) region of the CHO-RVLP genome. To determine the quantity of particles in a sample, threshold cycle (Ct) values are interpolated into a standard curve generated by a dilution series of an RNA standard (sRNA) provided in the kit (Figure 3). From those concentration values, RVLP LRVs for each experiment can be calculated.

Data indicate that the MockV RVLP kit can determine LRVs accurately — within ±0.5 of values

Table 2: Results from RVLP assay qualification (Target Conc. = target concentration, $\log_{10}$ RVLP/mL; Avg. Calc. Conc. = average calculated concentration, $\log_{10}$ RVLP/mL; SD = standard deviation; CV = coefficient of variation)

Target Conc.	Day	Avg. Calc. Conc.	SD	CV (%)	% to Target
9	1 (*n* = 4)	8.8	0.0	0.3	100%
	2 (*n* = 4)	9.1	0.1	1.1	
	1 and 2 (*n* = 8)	9.0	0.2	2.2	
8	1 (*n* = 4)	8.0	0.1	1.4	100%
	2 (*n* = 4)	8.0	0.2	1.9	
	1 and 2 (*n* = 8)	8.0	0.1	1.7	
7	1 (*n* = 4)	7.0	0.2	3.1	101%
	2 (*n* = 4)	7.2	0.0	0.4	
	1 and 2 (*n* = 8)	7.1	0.2	2.9	
6	1 (*n* = 4)	6.0	0.2	3.7	103%
	2 (*n* = 4)	6.3	0.1	2.2	
	1 and 2 (*n* = 8)	6.2	0.2	3.8	
5	1 (*n* = 4)	5.2	0.0	0.5	108%
	2 (*n* = 4)	5.6	0.2	2.9	
	1 and 2 (*n* = 8)	5.4	0.2	4.3	
4	1 (*n* = 4)	4.7	0.3	6.9	121%
	2 (*n* = 4)	5.0	0.1	1.8	
	1 and 2 (*n* = 8)	4.8	0.3	5.7	
3	1 (*n* = 4)	3.5	0.1	3.9	131%
	2 (*n* = 4)	4.3	0.1	3.1	
	1 and 2 (*n* = 8)	3.9	0.4	10.1	
2	1 (*n* = 4)	2.8	0.1	1.7	175%
	2 (*n* = 4)	4.0	0.1	2.8	
	1 and 2 (*n* = 8)	3.5	0.6	17.2	

Table 3: Summary of results from RVLP and XMuLV removal, expressed as log reduction values (LRVs); AEX = anion exchange, MMC = mixed-mode chromatography, ND = no data, NA = not applicable

Separation Method	RVLP LRV	XMuLV LRV	Δ LRV
Protein A (collaborator A)	1.70	2.25	–0.55
Protein A (collaborator B)	2.53	2.95	–0.42
Viral filtration	≥4.41	≥5.30	NA
Cation exchange	3.57	3.94	–0.37
AEX, flowthrough (centerpoint)	≥4.35	ND	NA
AEX, flowthrough (high condition)	1.31	ND	NA
AEX, bind–elute	≥3.96	ND	NA
MMC	≥4.42	≥6.65	NA

from XMuLV clearance data — across multiple modes of separation. To ensure reliable results, the precision, accuracy, and sensitivity of the kit's analytical assay were qualified according to ICH Q2(R1) (**16**).

Inter- and intraassay precision were determined by analyzing a 10-fold dilution series of RVLP stock solution run on consecutive days in quadruplicate. During intraassay analysis, low coefficients of variation (CVs) were achieved at each dilution point, indicating high levels of precision; however, interassay analysis revealed an increase in CVs as the analyte approached the low end of the dilution series (Table 2). Accuracy was determined by analyzing the degree to which the experimentally determined RVLP concentrations correlated with the expected concentrations at each dilution point. The data showed that high levels of accuracy were achieved for most points of the dilution series until the lower extreme was reached (Table 2).

As for sensitivity, an analytical method approaching its lower limit of quantification (LLoQ) will begin to diminish in precision and accuracy. In the case of the RVLP assay, interassay precision and accuracy began to diminish as the concentration of analyte approached 3.0 $\log_{10}$ RVLP/mL, and both parameters fell below acceptability at concentrations below 3.0 $\log_{10}$ RVLP/mL. Therefore, an LLoQ of 3.0 $\log_{10}$ RVLP/mL (10^3 RVLP/mL) was established.

RVLP Spiking Experiment Results: Table 3 provides results from RVLP spiking experiments conducted by several industry collaborators. Those data show a good correlation between RVLP- and XMuLV-derived LRVs across all tested modes of separation. When XMuLV removal was incomplete (e.g., during protein A and CEX separation), RVLP LRVs were slightly lower than in other cases, indicating that RVLPs can be considered as a worst-case model. When complete XMuLV removal was achieved (e.g., virus filtration and mixed-mode chromatography), complete RVLP removal was achieved as well. For AEX, comparable XMuLV data were unavailable; however, complete RVLP removal was anticipated during flowthrough at centerpoint conditions and during bind–elute operations, whereas a low LRV was expected when operating in flowthrough mode with a high load conductivity. Subsequent testing fulfilled those expectations, with complete clearance achieved during flowthrough at centerpoint and bind–elute conditions and with significant decreases in particle reduction during flowthrough at high conductivity (LRV = 1.31).

Results from RVLP spiking experiments conducted by several industry collaborators show a **GOOD CORRELATION** between RVLP- and XMuLV-derived log reduction values across all tested modes of separation.

MVM — A Relevant Worst-Case Model

MVM is a nonenveloped parvovirus that has developed a taste for CHO-cell bioreactors. Since 1985, seven of the 26 reported cases of bioreactor viral contamination have been attributed to MVM (**1**). That fact, coupled with the virus's small size (18–24 nm) and high tolerance to inactivation techniques (pH or chemical), makes it a relevant worst-case model for viral-clearance demonstration. As such, global regulatory agencies require proof of a process's ability to remove a parvovirus (typically MVM) before issuing clinical and commercial approvals.

The MockV MVM Kit: To use MVM in spiking studies, drug companies must overcome the same hurdles discussed above associated with enlisting a CRO to work with live virus. In 2020, Cygnus Technologies introduced the MockV MVM kit to address such barriers. Like its RVLP counterpart, the MVM kit provides process development scientists with a unique tool for conveniently generating viral-clearance data throughout a biopharmaceutical's life cycle, with applications possible for process

Spiking MVM-MVPs

Each MockV MVM kit contains a stock solution of noninfectious MVPs that mimic the physicochemical properties of MVM. The surrogate particles were produced by expressing the major capsid protein of MVM (VP2) in a recombinant system and then purifying the assembled particles. A published collaboration with the FDA compared the physical and chemical properties of the biosafety level 1 (BSL-1)–safe MVPs and live MVM (**17**). The results demonstrated that the surrogates' size, surface charge, and surface hydrophobicity were indeed similar to those of MVM (Figure 4). The physicochemical resemblance of the MVPs to MVM was confirmed again in a 2020 study published by scientists at Bristol Myers Squibb (**18**).

Figure 4: Transmission electron microscopy image of minute virus of mice (MVM) mock virus particles (MVPs)

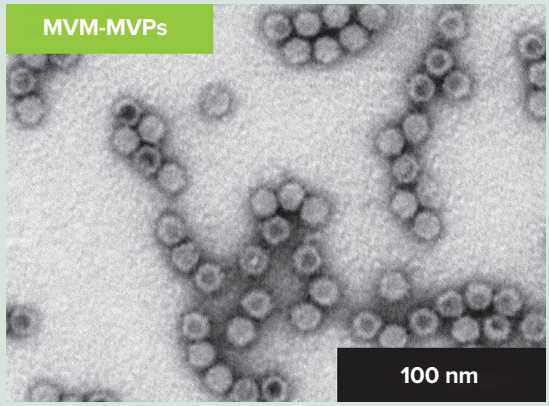
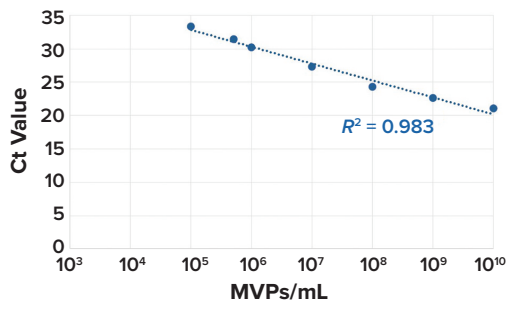


Table 4: Physicochemical data for live MVM and MVPs; * = relative hydrophobic affinity to phenyl (1.0 = insulin)

Analysis	Live MVM	MVM-MVPs
Hydrodynamic radii (multiangle light scattering)	18.4 ± 0.2 nm	17.2 ± 0.1 nm
Diameter (transmission electron microscopy)	24.6 ± 3.6 nm	25.6 ± 3.0 nm
Surface charge (pI)	5.99	5.81
Hydrophobicity*	0.28	0.35

Figure 5: MVP standard curve for immuno-qPCR assay; Ct = cycle threshold



development, characterization, and validation (through supplementation with prior knowledge).

Each MVM kit includes a vial of noninfectious MVM surrogates ("mock virus particles," MVPs), which can be handled safely on the benchtop (see the "Spiking MVM-MVPs" box, Figure 4, and Table 4); three 96-well plates for sample analysis; and all components required to perform an immuno-qPCR assay. By following the kit's protocol, scientists can detect as few as 10^5 MVPs/mL, enabling LRVs of ~4.0–5.0 to be determined. Each kit contains 1.5 mL of spiking MVPs at a concentration of 10^{12} RVLP/mL, which provides sufficient material for spiking up to 1,500 mL of load material to 0.1% (v/v) and for conducting analysis on 57 samples in triplicate in less than one day (each of the three plates provided can accommodate 19 samples). A real-time qPCR instrument is required along with standard laboratory equipment. Users need minimal experience with immuno-qPCR. Compatible purification steps include virus filtration and AEX, CEX, HIC, mixed-mode, protein A, and SEC chromatographies.

MVP Analytical Assay and Qualification: To analyze MVP concentrations in samples, the MockV MVM kit contains immunoassay and qPCR components. Samples are added to microwells coated with an anti-MVP capture MAb. After incubation and washing, a DNA-conjugated anti-MVP detector MAb is added. Following another incubation and washing step, a dissociation buffer is added to each well for five minutes. Then, 5 µL of sample is transferred from each well to a qPCR plate containing TaqMan primers/probes (Thermo Fisher Scientific) directed against the conjugated DNA. To determine the quantity of particles in samples, Ct values are interpolated into a standard curve generated by including a 10-fold dilution series of an MVP standard (Figure 5). From those concentration values, LRVs can be calculated.

Like the RVLP kit, the MockV MVM kit's analytical assay was qualified for precision and accuracy according to Q2(R1) guidelines (**16**). Intraassay precision was determined by analyzing a dilution series of spiking MVP (n = 3). Low CVs were achieved at each dilution point, indicating a high level of precision. Interassay precision was determined by analyzing the same dilution series as performed by two users (n = 6). Those data also

Table 5: Results from MVM-MVP assay qualification (Target Conc. = target concentration, $\log_{10}$ MVP/mL; Avg. Calc. Conc. = average calculated concentration, $\log_{10}$ MVP/mL; SD = standard deviation; CV = coefficient of variation)

Target Conc.	Day	Avg. Calc. Conc.	SD	CV (%)	% to Target
	1 (n = 3)	9.7	0.0	0.3	
10	2 (n = 3)	9.7	0.0	1.1	97%
	1 and 2 (n = 6)	9.7	0.0	2.2	
	1 (n = 3)	9.0	0.0	1.4	
9	2 (n = 3)	9.1	0.1	1.9	101%
	1 and 2 (n = 6)	9.1	0.1	1.7	
	1 (n = 3)	8.4	0.1	3.1	
8	2 (n = 3)	8.3	0.1	0.4	105%
	1 and 2 (n = 6)	8.4	0.1	2.9	
	1 (n = 3)	7.3	0.1	3.7	
7	2 (n = 3)	7.2	0.0	2.2	103%
	1 and 2 (n = 6)	7.2	0.1	3.8	
	1 (n = 3)	5.9	0.1	0.5	
6	2 (n = 3)	6.1	0.1	2.9	100%
	1 and 2 (n = 6)	6.0	0.1	4.3	
	1 (n = 3)	4.7	0.0	6.9	
5.7	2 (n = 3)	5.0	0.1	1.8	98%
	1 and 2 (n = 6)	4.8	0.1	5.7	
	1 (n = 3)	3.5	0.1	3.9	
5.0	2 (n = 3)	4.3	0.0	3.1	96%
	1 and 2 (n = 6)	3.9	0.0	10.1	

showed that low CVs were achieved (Table 5). Accuracy was determined by analyzing how the experimentally determined RVLP concentrations correlated with those expected at each dilution point. High levels of accuracy were achieved for all points of the dilution series (Table 5).

MockV MVM Kit Experimental Results

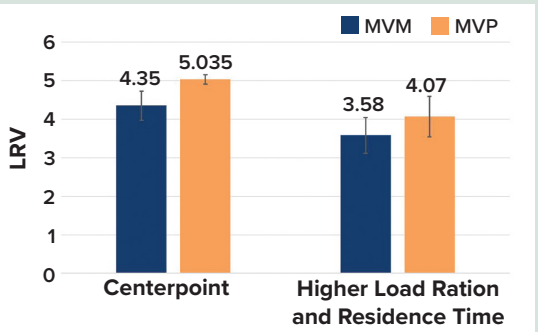
Table 6 summarizes all of the publicly available data from spiking studies performed with the MockV MVM kit. We highlight two of those studies below.

High-Throughput Screening of Chromatography Resins: Cygnus Technologies collaborated with the Vaccine Research Center of the US National Institutes of Health (NIAID-VRC) to perform high-throughput screening of different chromatography resins (**23**). Scientists evaluated the viral-clearance efficacy of multiple AEX resins using the MockV MVM kit across pH/conductivity conditions. Miniaturized columns for automated chromatography from multiple vendors were equilibrated with buffers containing 10 mM NaCl (pH 6.5, 7.5, 8.5); pH-adjusted loads containing a candidate vaccine were spiked to

Table 6: Summary of MockV MVM kit publications

Year	Collaborator	Description
2017	US Food and Drug Adminstration	Demonstrated MVPs' equivalent physicochemical characteristics to live MVM (**17**)
2018	Asahi Kasei	Showed equivalent removal and flux decay of MVM and MVPs by VF (**19**)
2019	Thermo Fisher	Showed equivalent removal of MVM and MVPs by HIC (**20**)
2019	AstraZeneca	Showed equivalent removal of MVM and MVPs by VF and AEX (**21**)
2020	Bristol Myers Squibb	Highlighted use of MockV MVM kit for protein A chromatography (**18**)
2021	Regenxbio	Showed equivalent removal of MVM and MVPs in a downstream process for AAV vectors (**22**)
2022	US National Institutes of Health	Demonstrated MVP clearance during high-throughput screenings (**23**)
2022	Penn State Univ.	Demonstrated MVP clearance during continuous processing (**24**)

Figure 6: Summary of MVM and MVP clearance by AEX



10^{11} MVP/mL and added to each column. The plate containing the columns was mixed and centrifuged while flowthrough 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 flowthrough was collected. All collected samples were analyzed for MVPs, and LRVs were calculated.

The data showed high variance in the resins' retention of MVPs as salt concentrations applied to the columns were increased (Table 7). Some resins retained MVPs even after exposure to moderate or high salt concentrations, whereas other resins did not. This case study exemplifies how the MockV MVM kit enables rapid collection of large amounts of viral clearance prediction data.

Assessing Viral Clearance During AAV Purification: As with MAb processes, viral clearance is an important aspect of gene therapy purification. The unique challenge for gene therapy processes is the

Table 7: Summary of high-throughput screening of MVM-MVP clearance by anion-exchange (AEX) chromatography resins, expressed as log reduction values (LRVs); FT = flowthrough

Resin (Process pH)	Load FT	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl	250 mM NaCl	300 mM NaCl	350 mM NaCl	500 mM NaCl	1 M NaCl
Toyopearl DEAE-650M (6.5)	4.80	4.66	1.40	0.64	0.63	0.63	0.63	0.63	0.63	0.63
Toyopearl DEAE-650M (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 (8.5)	4.80	4.66	4.55	2.01	0.77	0.74	0.73	0.73	0.73	0.73
POROS 50D (6.5)	4.80	4.66	4.55	0.75	0.69	0.67	0.67	0.66	0.66	0.66
POROS 50D (7.5)	4.80	4.66	4.55	3.18	0.94	0.86	0.84	0.83	0.82	0.82
POROS 50D (8.5)	4.80	4.66	4.55	1.19	0.94	0.87	0.87	0.86	0.86	0.86
POROS 50PI (6.5)	4.80	4.66	4.55	4.47	4.40	4.34	4.28	1.27	0.98	0.93
POROS 50PI (7.5)	4.80	4.66	4.55	4.47	4.40	4.34	4.28	3.95	0.73	0.71
POROS 50PI (8.5)	4.80	4.66	4.55	4.47	4.03	3.81	3.67	1.95	0.85	0.83
POROS 50HQ (6.5)	4.80	4.66	4.55	4.06	0.66	0.65	0.65	0.65	0.65	0.65
POROS 50HQ (7.5)	4.80	4.12	4.09	4.06	2.08	0.95	0.94	0.93	0.93	0.93
POROS 50HQ (8.5)	4.80	4.66	4.55	4.47	4.40	0.97	0.95	0.94	0.94	0.94
Cytiva Q SFF (6.5)	4.80	4.66	4.55	4.47	2.45	1.08	0.91	0.90	0.89	0.89
Cytiva Q SFF (7.5)	4.80	4.66	4.55	4.47	4.40	2.30	1.00	0.76	0.74	0.73
Cytiva Q SFF (8.5)	4.80	4.66	4.55	4.06	4.03	3.81	1.37	0.93	0.84	0.83
EMD Fractogel TMAE HiCap (6.5)	4.80	4.66	4.55	0.61	0.54	0.53	0.53	0.53	0.53	0.53
EMD Fractogel TMAE HiCap (7.5)	4.80	4.66	4.55	4.47	0.57	0.49	0.48	0.48	0.48	0.48
EMD Fractogel TMAE HiCap (8.5)	4.80	4.66	4.55	4.47	1.14	0.81	0.80	0.80	0.80	0.79
EMD Fractogel DEAE (M) (6.5)	4.80	4.66	4.55	1.87	0.79	0.77	0.77	0.77	0.77	0.73
EMD Fractogel DEAE (M) (7.5)	4.80	4.66	4.55	4.47	0.98	0.58	0.57	0.56	0.56	0.56
EMD Fractogel DEAE (M) (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 (6.5)	4.80	4.66	4.55	4.47	4.40	4.00	3.97	3.95	3.93	1.00
Toyopearl NH2-750F (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 (8.5)	3.80	3.78	3.76	3.75	3.73	3.61	3.60	3.59	3.58	1.08

product — e.g., adenoassociated virus (AAV). When the therapeutic vector is a virus or virus-associated particle, certain types of separation technologies commonly applied for MAbs (e.g., nanofiltration) are rendered useless. Regenxbio studied the efficacy of affinity- and AEX-based separations for viral clearance of a gene therapy using POROS AAVX resin from Thermo Fisher Scientific and monolith columns from BIA Separations (Sartorius), respectively (**22**). As part of that study, Regenxbio scientists used the MockV MVM kit in parallel with MVM spiking experiments. When using AAVX resin at centerpoint and worst-case operating conditions, MVP and MVM LRVs correlated strongly (Figure 6). In addition, worst-case parameters resulted in a ~1.0 $\log_{10}$ decrease in LRVs for both MVMs and MVPs. Similarly, data from monolith-column experiments showed a high correlation between MVP and MVM mass balance while operating under centerpoint and worst-case conditions. Overall, these experiments demonstrate the predictability of the MockV MVM kit for clearance of MVM during AAV purification steps.

Cygnus's MockV Analytical Services

Spiking-Study Sample Analysis: Analytical laboratories within biopharmaceutical companies often operate over capacity. There is little time to establish or test new assays. The Cygnus MockV team can ease staff burdens by analyzing samples generated by in-house MockV spiking study (RVLP or MVM). Both preliminary sample evaluation (spike/recovery studies) and spiking study analysis to determine LRVs will be performed within weeks of receiving your samples, and a detailed report is included with the service.

Custom CHO-RVLP Production: In addition to sample analysis, Cygnus offers custom services for CHO-RVLP production. The Cygnus MockV team will use CHO HCCF or protein A flowthrough from your upstream process to enrich endogenous RVLPs and

Instead of relying solely on a CRO-provided model retrovirus (XMuLV), downstream groups now can **INDEPENDENTLY** spike and assess the removal of the original retrovirus particle of regulatory concern.

generate a concentrated, purified stock solution for spiking applications. From a regulatory perspective, using the very RVLPs produced in an upstream process to validate the corresponding downstream purification process provides the most accurate insight into CHO-RVLP viral clearance.

Gaining Valuable Viral-Clearance Insights

Cygnus's BSL-1–compatible MockV kits enable you to gain viral-clearance insights for downstream process steps easily and economically in your own laboratory and on your own timeline. Why delay until late-phase clinical manufacturing before testing whether your downstream purification process provides sufficient viral clearance? Instead, take an actionable approach throughout process development and characterization. Recent updates to regulatory guidelines support the inclusion of prior knowledge. Using the MockV approach can enhance and supplement that valuable strategy. Instead of relying solely on a CRO-provided model retrovirus (XMuLV), downstream groups now can independently spike and assess the removal of the original retrovirus particle of regulatory concern.

To learn more about the MockV RVLP and MockV MVM kits from Cygnus Technologies, visit https://www.cygnustechnologies.com/MockV.

About Cygnus Technologies

Part of Maravai LifeSciences, Cygnus Technologies (https://www.cygnustechnologies.com) offers innovative solutions for viral-clearance assessment along with generic host cell protein (HCP) enzyme-linked immunosorbent assay (ELISA) kits for 23 different expression platforms, advanced orthogonal antibody coverage analysis services, Antibody Affinity Extraction with mass spectrometry (AAE-MS) identification of HCPs in process samples and drug substances, generic assay qualification services, and expert process-specific antibody and assay development services. The company's reputation for quality is recognized throughout the biopharmaceutical industry and among global regulatory agencies, with several of its generic HCP ELISA kits supporting numerous marketed biologics. Proprietary Cygnus technologies have been used to develop more than 100 process-specific antibodies and immunoassays for many global biopharmaceutical companies. Email techsupport@cygnustechnologies.com to discuss your current and future projects.

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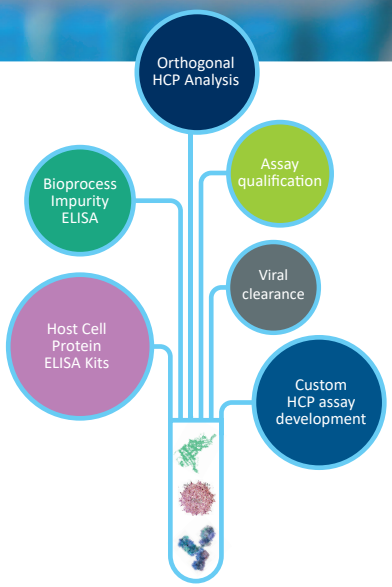

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