Predicting Viral Clearance: DOE, HTS and AAV Case Studies Utilizing a Non-Infectious MVM Surrogate During Downstream Development

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AAV collaboration with:

REGENXBIO | ThermoFisher SCIENTIFIC | Texcell

Introduction

Viruses can arise during the manufacture of biopharmaceuticals through contamination of exogenous viruses or endogenous expression of viral sequences. Regulatory agencies therefore require "viral clearance" validation studies for each biopharmaceutical prior to approval. These studies demonstrate the manufacturing process' ability at removing or inactivating virus and are conducted by challenging scaled-down manufacturing steps with a "spike" of live virus. These studies are conducted in BSL-2 facilities and are costly. Due to these hurdles, process knowledge pertaining to viral clearance is limited during development and characterization. The use of an accurate, economical and quantifiable non-infectious viral surrogate would enable downstream purification scientists to study viral clearance throughout process development.

A non-infectious *Minute Virus of Mice*—Mock Virus Particle (MVM-MVP) was generated by MockV Solutions, Inc. for use as an economical spiking surrogate. Discussed here are results from three studies. First, an AEX DOE study in which MVM-MVP clearance was compared to MVM and then used to generate a model for mapping design space. Second, a series of IEX high throughput screening experiments in which in-process vaccine material was spiked with MVM-MVP and processed through robo-columns under conditions of increased conductivity. Third, a comparative MVM vs. MVM-MVP clearance study utilizing AAVX resin (Thermo Fisher Scientific) in a downstream AAV process. The results from these studies demonstrate the value of utilizing this non-infectious tool for process development and characterization.

Particle Production

Minute Virus of Mice (MVM) was produced via acute infection of A9 cells and then purified utilizing ultracentrifugation, IEX and SEC (Texcell N.A.). Non-infectious MVM—Mock Virus Particles (MVM-MVP's) were assembled after expression in a baculovirus/Sf9 system. Particles were then purified via density centrifugation. Figure 1 shows Transmission Electron Microscopy (TEM) images of each particle.

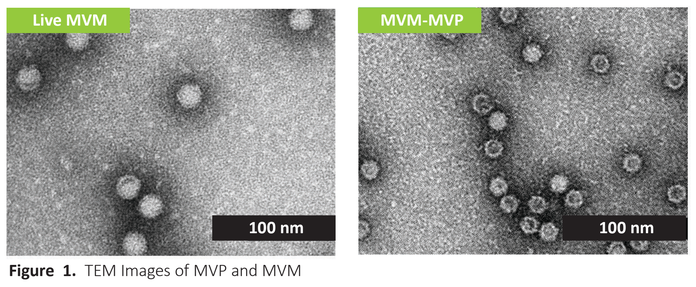


Figure 1. TEM Images of MVP and MVM

Physicochemical Comparison

Through a collaboration with the FDA, the physicochemical properties of MVMMVP were studied and compared to live MVM and PP7 bacteriophage (Johnson, 2017). For physical comparisons, TEM and Multi-angle Light Scattering (MALS) analyses were performed. For surface charge and hydrophobicity, each particle was analyzed via Chromatofocusing and Solute Surface Hydrophobicity techniques. Table 1 summarizes the results from these techniques.

Table 1. Physicochemical Comparison Summary

Analysis	Live MVM	MVM-MVP	PP7 Bacteriophage
Hydrodynamic Radii (MALS)	18.4 ± 0.2 nm	17.2 ± 0.1 nm	16.9 ± 0.4 nm
Diameter (TEM)	24.6 ± 3.6 nm	25.6 ± 3.0 nm	31.6 ± 1.6* nm
Surface Charge (pI)	5.99	5.81	4.74
Hydrophobicity**	0.28	0.35	0.61

* Reference value from Lute et. al. PDA J Pharm Sci Technol (2008)
** Relative hydrophobic affinity to Phenyl (1.0 = insulin)

Proof of Concept Studies

mAb AEX DOE (in collaboration with GlaxoSmithKline)

The objectives of this study was to confirm the utility of MVM-MVP for predicting MVM clearance by AEX and then to use the noninfectious particle in mapping the associated process design space through a design of experiments (DoE) study.

Q Sepharose FF was packed into 30 × 0.66 cm columns and qualified. A full-factorial, central composite face design of experiment (DoE) examining load pH and conductivity was constructed (Figure 2). Nonaxial-point runs 1–6 were spiked with MVM-MVP and live MVM (Texcell, N.A.) in parallel, whereas axial-point runs 7–10 were spiked only with MVM-MVP.

mAb containing in-process material (GSK) was adjusted to the pH/Cond. Conditions described above and spiked with either:

- 0.9% (v/v) MVM-MVP to a concentration of 9.95 log10 particles/mL, or
- 0.08% (v/v) live MVM to a concentration of 7.5 TCID50/mL

Run Number	Pattern	Load pH	Load Cond. (mS/cm)
1	- -	7.0	3.0
2	- +	7.0	13.0
3	00	7.5	8.0
4	00	7.5	8.0
5	+ -	8.0	3.0
6	+ +	8.0	13.0
7	+0	8.0	8.0
8	0-	7.5	3.0
9	-0	7.0	8.0
10	0+	7.5	13.0

Figure 2. AEX DOE

After sampling, 100 mL's of these spiked loads were processed through the Q-SFF column under standard conditions. A duplicate of each nonaxial MVM-MVP spiked experiment was performed. MVM-MVP and MVM samples were stored at –80 °C until analysis by Immuno-qPCR as discussed (above right) or by TCID50 infectivity assay, respectively. From these results, log-reduction values (LRV) were determined and compared (Table 2).

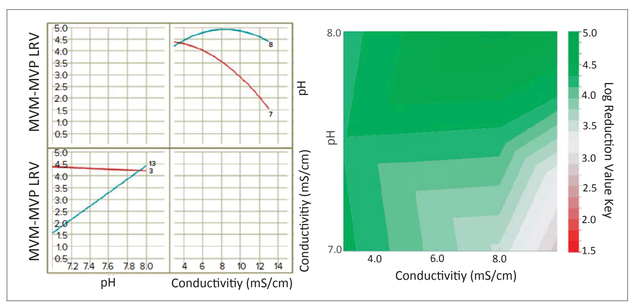
Table 2. LRV results from non-axial (A) and axial (B) runs

A

			Log Reduction Values	
Run	pH	Conductivity	MVM-MVP	MVM
AEX 1	7.0	3 mS/cm	4.17 ≥ 4.46	4.11
AEX 2	7.0	13 mS/cm	2.08 0.85	2.43
AEX 3	7.5	8 mS/cm	≥ 4.50	≥ 6.14
AEX 4 (20 CM)	7.5	8 mS/cm	≥ 4.41	4.27
AEX 5	8.0	3 mS/cm	≥ 4.28 ≥ 4.32	≥ 5.99
AEX 6	8.0	13 mS/cm	≥ 4.37 ≥ 4.57	3.24

B

Conditions	MVM-MVP LRV
AEX 7 — pH 8, 8 mS/cm	≥ 4.21
AEX 8 — pH 7.5, 3 mS/cm	≥ 4.21
AEX 9 — pH 7, 8 mS/cm	≥ 3.01
AEX 10 — pH 7.5, 13 mS/cm	3.46



A backwards stepwise regression was used for data analysis and model building. Each model's statistical significance was evaluated using ANOVA and effect's test. The quality of the model was determined by r^2 values and lack of fit tests. All inequalities (assay LOQ limits) were taken as actual values. Astatistically significant and valid model from the data set (r^2 = 0.92 $p < 0.01$, no lack of fit). A two-d mensional response surface graph and interaction plot (Figure 3) were drawn to show the general trend of LRV outcomes when operating with different load pH and conductivity parameters. The results demonstrate that load pH strongly influences load conductivity's effect on LRV.

Figure 3. Interaction plot **(left)** and design-space process map **(right)** illustrating the effects of pH and conductivity on MVM-MVP clearance

IEX HTS Studies (in collaboration with NIH NIAID-VRC)

The Purpose of this study was to utilize MVM-MVP to screen performance of AEX and CEX resins from several vendors across a range of pH/Cond conditions in high throughput screening mode.

Robocolumns were equilibrated with buffer containing 10 mM NaCl (pH 6.5, 7.5, 8.5 for AEX; pH 5.5, 6.5 for CEX). pH adjusted load (vaccine) was spiked to 1E11 MVM-MVP/mL and added to each column. The plate was mixed and centrifuged while unbound flow through was collected. A series of increasing NaCl concentrations were added to the columns and after each addition, the plate was mixed, centrifuged and sample were collected. All samples collected were analyzed for MVM-MVP and LRV's were determined (Figure 4).

A

AEX Resin Screen	Load FT	50mM NaCl	100mM NaCl	150mM NaCl	200mM NaCl	250mM NaCl	300mM NaCl	500mM NaCl	1M 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	0.74	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

B

CEX Resin Screen	FT/Chase	50mM NaCl	100mM NaCl	150mM NaCl	200mM NaCl	250mM NaCl	300mM NaCl	500mM NaCl	1M NaCl Strip
Resin 1, pH 5.5	3.80	1.57	0.80	0.77	0.75	0.74	0.74	0.73	0.72
Resin 1, pH 6.5	2.74	1.68	0.49	0.47	0.46	0.46	0.46	0.46	0.46
Resin 2, pH 5.5	4.80	1.83	0.40	0.39	0.39	0.38	0.38	0.38	0.38
Resin 2, pH 6.5	2.61	2.36	-0.08	-0.09	-0.09	-0.09	-0.09	-0.09	-0.09
Resin 3, pH 5.5	3.03	0.58	0.48	0.47	0.47	0.47	0.47	0.47	0.46
Resin 3, pH 6.5	1.66	1.35	0.55	0.54	0.53	0.53	0.53	0.53	0.53
Resin 4, pH 5.5	4.80	1.09	0.99	0.97	0.95	0.94	0.93	0.92	0.87
Resin 4, pH 6.5	1.35	1.06	0.67	0.64	0.63	0.63	0.63	0.63	0.63
Resin 5, pH 5.5	1.95	1.01	0.95	0.91	0.88	0.86	0.85	0.82	0.77
Resin 5, pH 6.5	1.22	0.76	0.70	0.69	0.69	0.69	0.69	0.68	0.68
Resin 6, pH 5.5	2.54	0.69	0.55	0.54	0.54	0.54	0.53	0.53	0.52
Resin 6, pH 6.5	1.23	1.12	0.52	0.50	0.50	0.50	0.50	0.50	0.50
Resin 7, pH 5.5	2.29	1.41	0.83	0.80	0.79	0.78	0.78	0.78	0.77
Resin 7, pH 6.5	2.81	1.98	1.02	0.98	0.97	0.96	0.96	0.96	0.95
Resin 8, pH 5.5	0.94	0.72	0.70	0.70	0.69	0.69	0.69	0.69	0.68
Resin 8. pH 6.5	1.46	0.51	0.47	0.47	0.47	0.47	0.47	0.47	0.47

Figure 4. LRV results from each fraction collected during anion **(A)** and cation **(B)** exchange HTS studies

Quantification

Samples from all experiments were analyzed for MVM-MVP quantity via Immuno-qPCR (Figure 5).

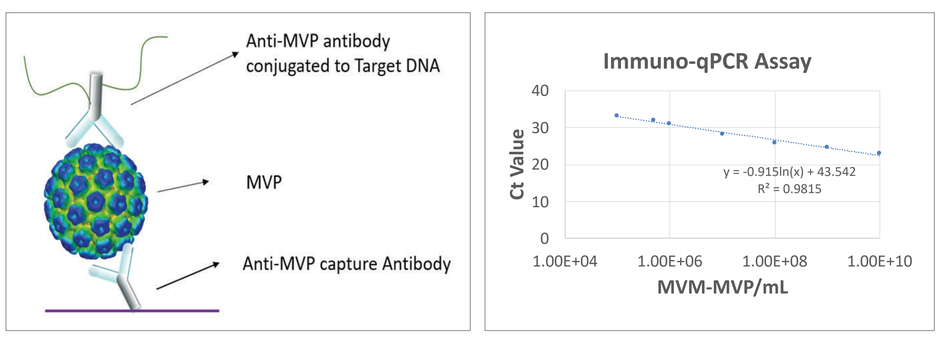


Figure 5. Immuno-qPCR Standard Curve.

AAVX Study (in collaboration with REGENXBIO, Thermo Fisher Scientific and Texcell)

The Purpose of this study was to understand the viral clearance potential of Thermo Fisher Scientific's AAVX resin in a representative downstream AAV process and to determine the predictive ability of utilizing MVM-MVP's.

AAVX resin was packed into columns (5 mL CV) and qualified. "Centerpoint" and "Worst Case" runs were conducted according to REGENXBIO process parameters (Table 3). In addition, runs with an alternate AAVX ligand were performed at centerpoint. For each run, in-process AAV material, provided by REGENXBIO, was spiked with either MVM-MVP (to a target 10.0 log10 MVP/mL), MVM or XMuLV.

For centerpoint runs, 150 mL's of spiked material was loaded, for worst case runs, 200 mL's was loaded. Samples were collected throughout each process phase and stored at -80 °C prior to Immuno-qPCR or TCID50 analysis. From these results, LRV's were determined and compared (Table 4,5 and Figure 6).

Table 4. Immuno-qPCR Standard Curve.

		Total log_{10} MVP	% of MVP	LRV
Run 1 Centerpoint	Load	12.3		
	FT	12.0	52.6%	
	Wash 1	10.0	0.5%	
	Benzonase Wash	11.3	10.4%	
	Wash 2	8.7	0.1%	
	Elution	7.4	0.0%	4.91
	CIP	6.9	0.0%	
Run 2 Centerpoint	Load	12.2		
	FT	12.0	67.0%	
	Wash 1	9.7	0.3%	
	Benzonase Wash	11.2	10.4%	
	Wash 2	8.7	0.0%	
	Elution	7.0	0.0%	5.16
	CIP	6.7	0.0%	
Higher Load Ratio + Residence Time	Load	11.9		
	FT	11.8	79.1%	
	Wash 1	9.9	1.1%	
	Benzonase Wash	11.0	14.3%	
	Wash 2	9.0	0.1%	
	Elution	7.8	0.0%	4.07
	CIP	6.8	0.0%	
Alternate Ligand	Load	11.8		
	FT	11.7	74.0%	
	Wash 1	9.8	1.0%	
	Benzonase Wash	10.3	3.2%	
	Wash 2	6.6	0.0%	
	Elution	7.6	0.0%	4.28
	CIP	6.7	0.0%	

Table 5. XMulV and MVM Clearance Results.

		Total Virus (log_{10})		% of Virus		LRV	
		XMuLV	MVM	XMuLV	MVM	XMuLV	MVM
Center-point	Load	9.92	8.18				
	FT	10.14	7.92	165.8%	54.9%		
	Wash 1	8.41	6.10	3.1%	0.8%		
	Benzonase Wash	7.73	5.37	0.7%	0.2%		
	Wash 2	6.84	4.68	0.1%	0.0%		
	Elution	<3.48	3.75	0.0%	0.0%	>6.43	4.35 ± 0.38
	CIP	<4.12	4.98	0.0%	0.1%		
Higher Load Ratio + Residence Time	Load	9.72	7.89				
	FT	9.62	7.63	79.3%	54.8%		
	Elution	5.09	4.31	0.0%	0.0%	4.63	3.58 ± 0.46
Alternate Ligand	Load	9.37	7.94				
	FT	9.48	8.40	127.8%	285.8%		
	Wash 1	8.47	6.42	12.5%	3.0%		
	Benzonase Wash	7.61	5.13	1.7%	0.2%		
	Wash 2	6.95	3.88	0.4%	0.0%		
	Elution	4.29	4.00	0.0%	0.0%	5.08	3.79 ± 0.39
	CIP	7.53	4.66	1.4%	0.1%		

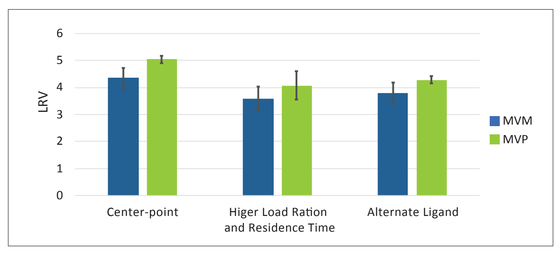


Figure 6. MVP vs. MVM LRV Comparison.

Summary

- Non-infectious MVM-MVP's mimic the physicochemical properties of MVM
- MVM-MVP was successfully used as spiking surrogate for AEX DOE, IEX HTS, and AAV studies
- AAVX resin effective viral clearance removal step in AAV process

High Value

- 10 study MVM-MVP DOE consumed 1 Kit ($4,000) and took 1 week to execute and analyze
- All experiments performed in-house (BSL-1)

References

1. John S.,, K.A., Frey, D.D. et al. Appl Biochem Biotechnol (2017) 183:318
2. Lute, S. Brorson, Riordan,W., Pease, L. F., Tsai, D. H., Levy, R., Haque, M., Martin, J., Moroe, I., Sato, T., Morgan, M., & Krishnan, M. (2008). A consensus rating method for small virus-retentive filters. I. Method development. PDA Journal of Pharmaceutical Science and Technology, 62, 318–333.

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