Predicting Viral Clearance at Your Benchtop

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Viral contamination is an inherent risk during the manufacture of therapeutic products such as antibodies, vaccines, viral vectors, and plasma derivates. Whether introduced endogenously from raw materials or exogenously through manufacturing operations, unmitigated viral contaminations can lead to serious health implications and plant shutdowns. Therefore, international regulatory agencies require sponsoring companies to validate the "viral clearance efficacy" of their individual downstream purification process steps before clinical trials or commercial approval.

VIRAL CLEARANCE TESTING

Viral clearance validation is assessed through small-scale "spiking studies," where model mammalian viruses (e.g., minute virus of mice [MVM]) are introduced into in-process material that is then processed through a purification technique (e.g., chromatography, nanofiltration, and low pH). Viral quantity pre/post-processing is determined through an infectivity (e.g., TCID50) or qPCR assay and the log reduction value (LRV) is calculated. These studies require specialized Biological Safety Level (BSL) laboratories and experienced personnel, resulting in costs that can soar well over US$100,000. These hurdles deter many companies from analyzing viral clearance during the years of small-scale process development that lead to validation. Instead, such companies spend considerable resources optimizing their manufacturing processes before gaining knowledge of viral clearance efficacy. Unfortunately, that increases the risk of validation failure, forcing biomanufacturers to invest additional time and money redeveloping some process steps—which can, in turn, postpone regulatory approval.

NEW TIME-SAVING APPROACHES

In 2020, Cygnus Technologies introduced the MockV™ MVM Kit, a BSL-1 compatible viral clearance prediction tool that includes a non-infectious mock virus particle (MVP) that mimics the physicochemical properties of live MVM. The Kit also contains all the necessary quantification components to determine MVP-LRV, thus enabling downstream purification scientists to easily and economically conduct viral clearance assessments in the comfort of their own laboratory.

Through a collaboration with the FDA, the physicochemical properties of this MVM-MVP were first studied and compared with live MVM. The results from this study demonstrated comparable size, surface charge, and surface hydrophobicity properties among MVM-MVP and MVM (**Figure 1, Table I**) (1). To quantify MVM-MVP in solution, an Immuno-qPCR assay was optimized (**Figure 2**). This assay enables MVM-MVP quantification over a 4.0 $\log_{10}$ dynamic range in less than 5 hours. Data demonstrating the LRV's comparability with MVM achieved through the use of the Kit have been generated for a variety of downstream applications including; nanofiltration (2), anion exchange chromatography (3-4), hydrophobic interaction chromatography (5), and AAV

Figure 1. Live MVM versus MVM-MVP.

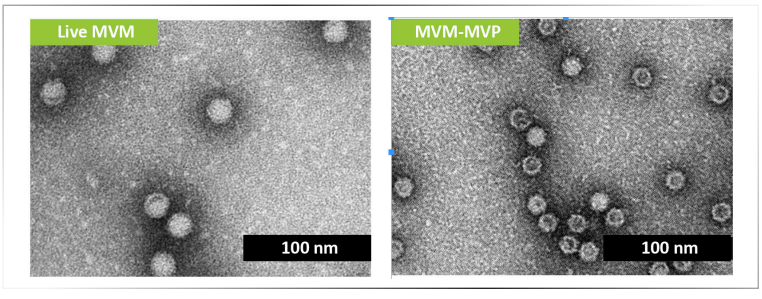

Table I. Comparing live MVM, MVM-MVP, and PP7 Bacteriophage.

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 Change (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)

affinity chromatography. The Kit has also been utilized to provide viral clearance output for Design of Experiments (**Figure 3**) and High Throughput Screening (HTS) applications.

During a high-throughput screening (HTS) collaboration with the National Institute of Health's (NIH) Vaccine Research Center (NIAID-VRC), the viral clearance performance of several AEX and CEX resins (from multiple vendors) were screened with MVM-MVP across a range of pH/conductivity conditions. 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 loads (vaccine) were 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 was added to the columns and after each addition, the plate was mixed, centrifuged and samples were collected. All samples collected were analyzed for MVM-MVP and LRVs were determined (**Figure 4**). The information gathered from these experiments enabled the NIH to select several resins for further process development activities. Obviously, the Tecan used for these experiments could not be transported to a viral clearance facility for live viral clearance experiments, nor could live virus be shipped for use by the NIH in-house. Thus, the MockV™ MVM Kit provided a unique and economic opportunity to gain viral clearance prediction in a HTS manner.

Figure 2. Immuno-qPCR assay.

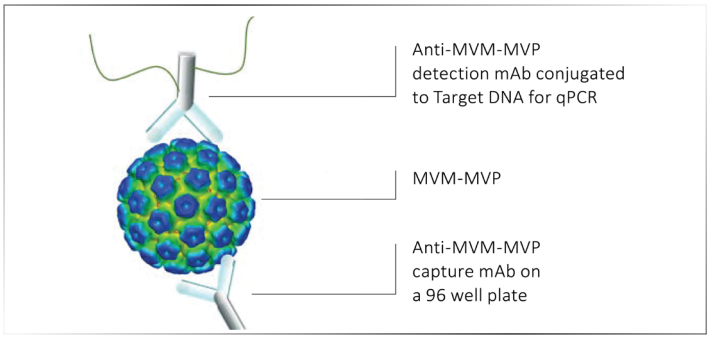

Figure 3. Viral clearance output for Design of Experiments.

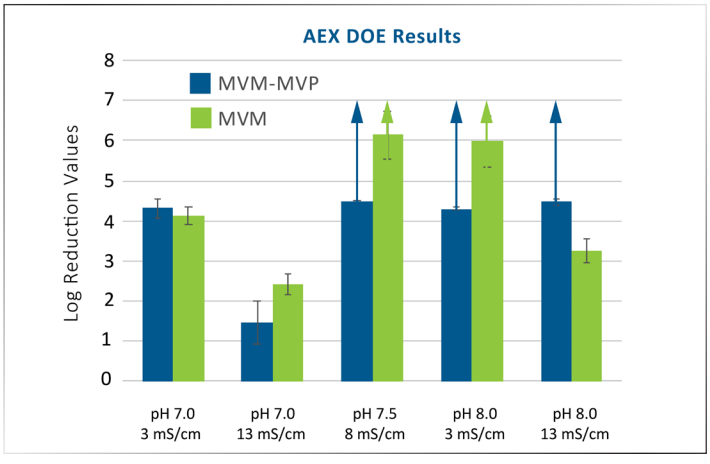

Figure 4. AEX and CEX resin screen results.

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

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