A Multi-spike Viral Clearance Approach

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Abstract

The ICH Q5A(R2) guidelines state that products derived from cell lines must demonstrate virus removal or viral inactivation through established purification processes. This requirement must be completed prior to filing a new drug application and the commercial phase. A mono spike of an individual test material is the traditional method to evaluate purification steps in a viral clearance study. The approach we present here has multiple viruses spiked simultaneously into one test material and are evaluated in a test system specific to each virus. The multi-spike requires less test material as well as shortens the length of a viral clearance study. The approach maintains the same data integrity that is achieved when mono-spike studies are utilized.

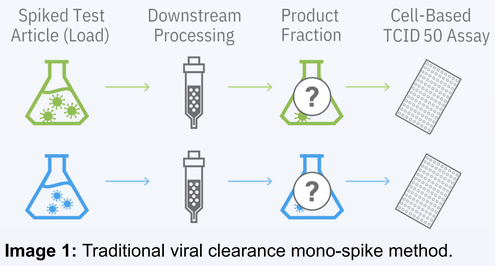

Image 1: Traditional viral clearance mono-spike method.

Background

The most significant bottleneck in drug development is the viral clearance study. These studies require substantial time and materials prior to IND and BLA filings. A mono-spike approach, which is the traditional viral clearance approach, evaluates one virus per experiment, increasing these needs across a full study. The multi-spike approach, in which two viruses are spiked into the test material simultaneously, addresses the limitations of a traditional study, while keeping data integrity.

Objective

To evaluate the multi-spike assay as an alternative to a tradition mono-spike viral clearance assays for AAV and mAbs.

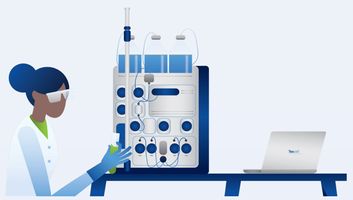

Image 1: An AKTA for performing viral clearance studies.

Materials and Methods

Experiments were set up in triplicate to evaluate multi-spiking the combinations. Three different test materials were used for each set of experiments. The test materials were spiked with a known volume of each of the viruses in the combination (e.g. 0.5 mL of XMuLV and 0.5 mL of MVM). Samples were taken and tested via a 50% Tissue Culture Infectious Dose ($TCID_{50}$) assay in each of the viruses' indicator test system at an appropriate dilution to capture the titer of the virus. For each virus, a control assay was set up.

Test Material	Virus	Spike Volume (mL)	Indicator Cell Line
Test Material	MVM	0.5	324K
	XMuLV	0.5	PG-4
Test Material	PPV	0.5	PT-1
	HSV-1	0.5	Vero
Test Material	Ad5	0.5	A549
	BVDV	0.5	BT
Test Material	Reo-3	0.5	HEK293
	BVDV	0.5	BT
Test Material	XMuLV	0.5	PG-4
	MVM	0.5	324K
	SV40	0.5	CV-1

Table 1: Shows the set up of the R&D experiments.

There are other options for virus combinations offered:

- XMuLV and SV40
- Hepatitis A Virus (HAV) and XMuLV
- SV40 and MVM
- PPV and HAV

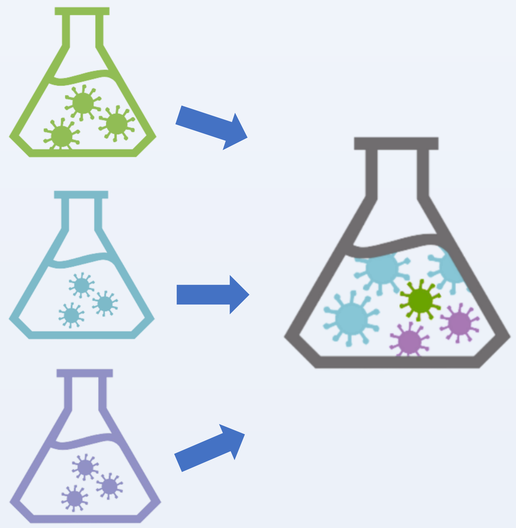

Image 3: Example of a triple-spike of XMuLV, MVM, and SV40.

Results

The results tables show the average of each multi-spike experiment and are reported in log_{10} value. The multi-spike assays are compared to the control assay results.

***** Validated Assay *****

Test Material	Virus	Control Virus	Multi-Spike Virus	Difference
1	MVM	4.99	4.82	0.17
	XMuLV	4.99	4.82	0.17
2	MVM	4.91	5.01	0.10
	XMuLV	5.23	5.25	0.02
3	MVM	4.67	4.93	0.26
	XMuLV	4.67	4.87	0.20

Table 2: Shows the observed differences are less than 1 log.

Test Material	Virus	Control Virus	Multi-Spike Virus	Difference
1	PPV	7.66	7.35	0.31
	HSV-1	4.83	4.83	0.00
2	PPV	7.47	7.37	0.10
	HSV-1	4.91	5.01	0.10
3	PPV	7.47	7.59	0.12
	HSV-1	4.83	4.80	0.03

Table 3: Shows the observed differences are less than 1 log.

Test Material	Virus	Control Virus	Multi-Spike Virus	Difference
1	BVDV	5.15	5.20	0.05
	Ad5	5.35	6.10	0.75
2	BVDV	5.47	5.46	0.01
	Ad5	6.31	5.89	0.42
3	BVDV	5.70	5.41	0.29
	Ad5	5.83	5.46	0.37

Table 4: Shows the observed differences are less than 1 log.

Test Material	Virus	Control Virus	Multi-Spike Virus	Difference
1	BVDV	5.39	5.86	0.47
	Reo-3	5.39	5.70	0.31
2	BVDV	4.67	5.62	0.95
	Reo-3	5.23	5.25	0.02
3	BVDV	5.94	5.62	0.32
	Reo-3	5.86	5.25	0.61
4	BVDV	5.39	5.49	0.10
	Reo-3	5.07	5.09	0.02

Table 5: Shows the observed differences are less than 1 log.

Test Material	Virus	Control Virus	Multi-Spike Virus	Difference
1	MVM	8.02	7.93	0.09
	XMuLV	4.75	4.51	0.24
	SV40	6.43	6.61	0.18
2	MVM	7.39	7.53	0.14
	XMuLV	4.91	4.67	0.24
	SV40	6.83	6.56	0.27
3	MVM	5.15	4.59	0.56
	XMuLV	5.07	5.02	0.05
	SV40	6.51	6.69	0.18

Table 6: Shows the observed differences are less than 1 log.

Conclusions

The industry is trending to reduce the costs of therapeutics to patients. The multi-spike assay lends itself to reducing the materials required, the number of runs for execution, and technical time by a factor of a factor of 20% or more, depending on the scope of the project. This assay supports the new ICH Q5A (R2) reference regarding the Q2 (R2) and complementary Q14 guidelines.

The multi-spike has many aspects that will streamline a viral clearance process. Viral clearance studies can be lengthy, and spiking with two or more viruses would cut the time of study execution. The volume of test material needed would be less than the volume needed for a traditional study. This would be beneficial for AAV products due to low availability of test material. mAbs would also see a reduction in materials as amount of chromatography runs would decrease.

The XMuLV and MVM validated multi-spike assay will contribute to the evolving landscape of both early and late phase activities. There were challenges encountered during the development of multi-spike, such as virus interference with each other and their indicator system. The validation of our R&D multi-spike panel will add to this new landscape of viral clearance.

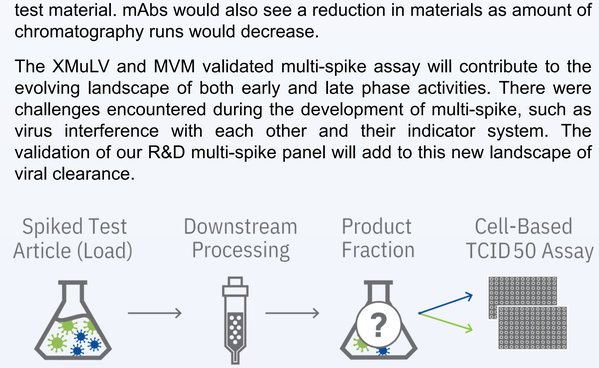

Image 4: A validated XMuLV and MVM multi-spike method for viral clearance studies.

References

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