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Contract Testing Services

Application Note

Application of a Dual Spiking Strategy in Viral Clearance Studies

Introduction

The viral safety of biopharmaceutical drugs is paramount to support patient safety. No single approach is sufficient to ensure safety, and a multifaceted approach is taken, often referred to as the viral safety tripod shown below (**Figure 1**).



PREVENT – Selection and characterization of the cell line and raw materials used in the process.

DETECT – Implement testing of the product at appropriate stages in the manufacturing process.

REMOVE – The capacity of the downstream process to remove or inactivate potential viral contamination.

Figure 1: Viral Safety Tripod

This technical note will discuss an alternative spiking strategy that can be applied in viral clearance studies to reduce the time taken to complete the spiked runs and reduce the sampling burden from the clinical run feeding the viral clearance study.

Spiking Studies

For products expressed from well characterized cell lines (e.g., rodent cell lines such as CHO and Sp2/0), a viral clearance study is required to support the initiation of clinical trials (EMEA/CHMP/BWP/398498/2005). These studies evaluate murine leukaemia retrovirus (MLV) and minute mouse virus (MMV).
A summary of characteristics is displayed in **Table 1**.

Xenotropic Murine leukaemia virus (MLV) is selected as a specific model virus and is a model for the retroviral contamination known to be present in many rodent cell lines. Minute mouse virus (MMV) is selected as a non-specific model virus representing a severe challenge of the downstream process.

Parvoviruses are very stable in the environment and resistant to inactivation by gamma-irradiation and other physical treatments. They pose a severe challenge to nanofiltration systems because of their small size of ~18 to 24 nm. Once the manufacturing process has been finalized, viral removal is evaluated in accordance with ICH Q5A(R2).

Table 1: Summary of MLV and MMV Characteristics

Virus	Strain	Genome	Envelope	Family	Size (nm)	Resistance to Physical /Chemical Reagents
MLV	pNFS Th-1	ssRNA	Yes	Retro	80–110	Low
MMV	Prototype	ssDNA	No	Parvo	18–24	Very High

A critical component in viral clearance studies is the use of “representative material” in the spiking studies. To support this, samples are typically taken from a GMP batch. Before Investigational New Drug (IND) application, a single batch is typically manufactured to generate sufficient material to initiate clinical trials. Strategies to reduce the overall timeline for the viral clearance study is critical to ensure that the viral clearance study is not on the critical path for the regulatory submission. One strategy designed to reduce the timeline for the viral clearance study is the implementation of a multi-spike approach.

A typical purification process will include 2 to 3 chromatography steps, and 2 steps designed specifically to inactivate or remove virus (**Figure 2**). Typically, removal of both MLV and MMV is evaluated for the chromatography steps, MLV only for the inactivation step and, with the 2023 update to ICH Q5A(R2), the option to evaluate MMV only for the VRF step, extrapolating the data to MLV.

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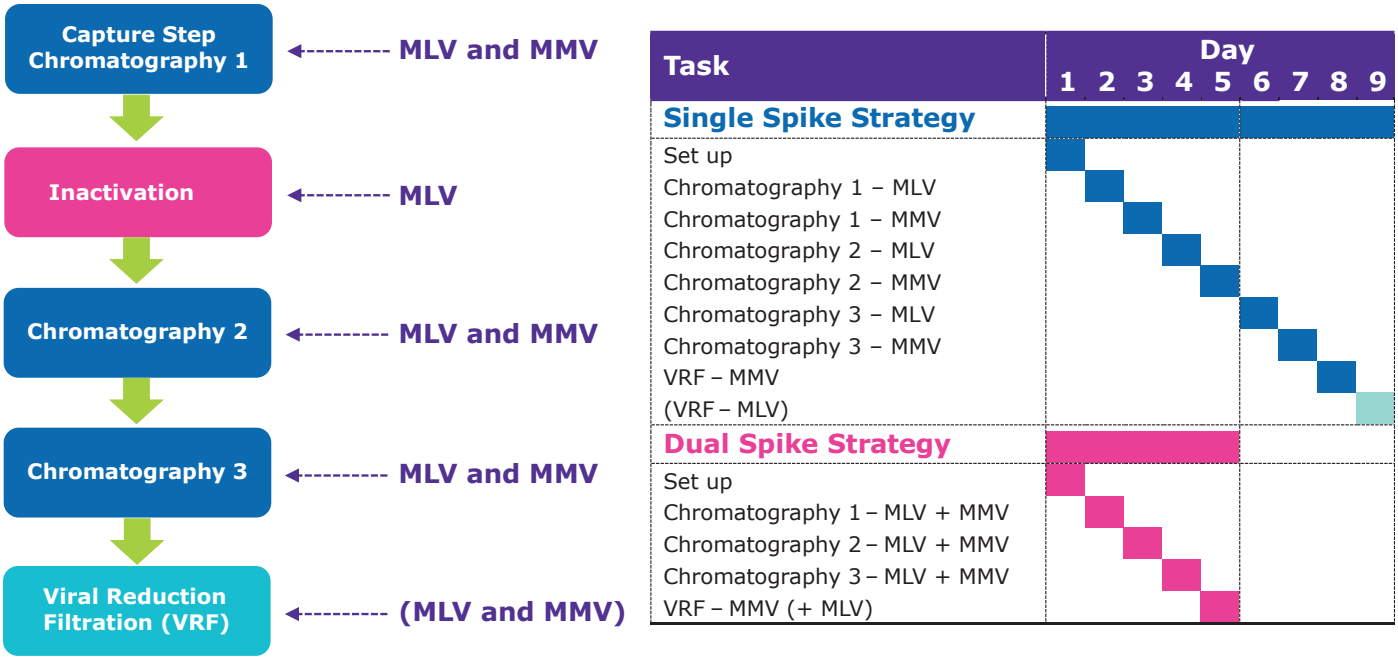


Figure 2: Typical Process and Spiking Strategy

Besides decreasing the time needed for the spiked runs, employing a dual spiking approach offers further benefits:

- Less product is required to support the spiked runs, resulting in more filled product released for clinical trials.
- A reduction in the number of documents within the study file, decreasing the time required for review and audit of the data.
- A reduction in the number of columns and or membrane devices required to complete the spiked runs.
- A reduction in the volume of buffers required to support the study.
- A reduction in waste for disposal (solid and liquid waste).

Feasibility Study

The multi-spike approach has been applied previously using molecular end point assays, exploiting the specificity of the molecular primers and probes used in the assays (Valera et al, 2003 and Lute et al, 2009). However, applying the same approach using infectivity assays has been more challenging with cellular tropism lacking the same specificity seen in the molecular end point assays.

A preliminary study was completed to evaluate the potential to apply a dual spiking approach for MLV and MMV to reduce elapsed time to complete an IND-enabling viral clearance study prior to moving into a validation phase. This preliminary study evaluated the following:

- Cell line specificity
- Spike recovery
- Interference

Cell Line Specificity

- No cytopathic effect was observed inoculating MLV onto 324 K cells, the cell line used in the MMV infectivity assay.
- No cytopathic effect was observed inoculating MMV onto PG-4 cells, the cell line used in the MLV infectivity assay.

Spike Recovery

Preparations of MLV and MMV to reflect viral titres in spiked test article (5.5 Log_{10}/ml) and product from an effective step (~1.0 Log_{10}/mL) were spiked with 0.1% v/v MLV (into MMV preparations) and 0.1% MMV (into MLV preparations).

- MMV Spike Recovery – full recovery in both 5.5 and 1.0 Log_{10}/mL MLV. Titre was within 0.17 Log_{10}/mL of the spiked positive control.
- MLV Spike Recovery – full recovery in both 5.5 and 1.0 Log_{10}/mL MMV. Titre was within 0.18 Log_{10}/mL of the spiked positive control.

Interference

Stock virus (MLV or MMV) was titrated through 5.5 and 1.0 Log_{10}/mL (MMV or MLV). The titre of each stock was within 0.67 Log_{10}/mL of the certified batch titre.

- MLV titrated through 5.5 Log_{10}/mL MMV – reported titre was within 0.35 Log_{10}/mL of the certified batch titre and assay positive control.
- MLV titrated through 1.0 Log_{10}/mL MMV - reported titre was within 0.09 Log_{10}/mL of the certified batch titre and assay positive control.
- MMV titrated through 5.5 Log_{10}/mL MLV - reported titre was within 0.67 Log_{10}/mL of the certified batch titre and assay positive control.
- MMV titrated through 1.0 Log_{10}/mL MLV - reported titre was within 0.61 Log_{10}/mL of the certified batch titre and assay positive control.

Validation

The infectivity assay used to evaluate samples generated from dual spiked samples is unchanged from that used in conventional single spiked studies. The key difference is the input sample is known to contain both MLV and MMV. The infectivity assay was challenged with combinations of high titre (HT), medium high titre (MHT), medium titre (MT), and low titre (LT) MLV and MMV preparations, testing precision, accuracy reproducibility, and robustness. The validation confirmed the assay was robust within the typical titre range anticipated in spiked samples.

Table 2: Assay Robustness

	Difference from nominal Titre (Log_{10} $TCID_{50}$/mL)	
Sample Composition MLV:MMV	MLV	MMV
HT:HT	●	●
HT:LT	●	●
LT:HT	●	●
LT:LT	●	●
MT:MT	●	●
LT:MT	●	●
LT:MHT	●	●

● Greater than 1 Log_{10} difference

● Within 1 Log_{10}/mL

Conclusions

Dual spiking has the potential to reduce both the timeline for spiking studies and the burden of sampling from clinical manufacturing batches.

The validation activities identified some limitations with respect to the maximum MMV titre in dual spiked samples, however, the maximum titre determined was within normal working ranges.

Spiking strategies should be considered to maximise potential log reduction factors where an effective step is being evaluated balancing the ratio of each virus spike without exceeding the upper 5% v/v total typically applied to studies.

Robust pre-study testing will be required for each study to confirm there are no product related influences that impact the detection of both viruses in a dual spiked sample.

References

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MS_TN14950EN Ver. 1.0 66805 02/2026