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Virus clearance from conventional to unconventional process steps in Biologics

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Introduction

The risk of virus contamination is common to all biologicals whose production involves the source material of animal or human in origin. To assure the safety of mammalian cells derived products, it is necessary for manufacturers to evaluate their purification processes by spiking with model viruses to show removal or inactivation of several logs of viral infectivity.

The choice and number of viruses that may be used in a viral clearance study are dictated by the nature & origin of the production cell line, as well as the nature & origin of the animal-derived materials used in production and purification

In general, at least two viruses, one enveloped (typically a retrovirus, e.g., MuLV) and one non-enveloped (preferably a parvovirus, e.g., MVM), are used in the early clinical phases of product development. Three or more viruses may be used to generate data for registration-enabling studies.

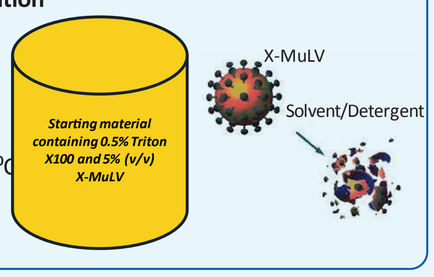
Rationale for virus clearance study using unconventional process steps

- The objective of viral clearance studies is to assess the process steps that can be considered to be effective in inactivating/removing viruses and to quantitatively estimate the overall level of virus reduction obtained by the process.
- Generally, conventional downstream purification steps such as Affinity Chromatography, Low PH Inactivation, AEX Chromatography and Nanofiltration will be used across Biopharmaceuticals for virus safety evaluation.
- At Syngen's Virus Testing Facility, virus safety evaluation was assessed for unconventional process steps such as Solvent/Detergent Inactivation, Depth Filtration and CEX Chromatography using enveloped (X-MuLV) and non-enveloped (MVM) viruses, to identify if additional capacity of virus clearance can be built into the purification process.

Module 1: Virus inactivation by SD treatment

Viral inactivation studies by solvent/detergent inactivation

- pH adjusted starting material was spiked with 5% (v/v) of X-MuLV stock solution.
- Added solvent/detergent: 0.5% Triton X100
- The spiked material's incubation temperature: 20.0±0.5°C
- Incubation time: 60 minutes and 120 minutes



Viral inactivation studies by solvent/detergent inactivation

Table 1: Solvent/detergent inactivation reduction factor: X-MuLV

Sample Description	Log_{10} adjusted Viral Titer	Log_{10} Reduction
Load Control	6.77	NA
Processing Control	6.60	NA
Solvent/detergent inactivation sample T=60 Minutes	<3.20	>3.57
Solvent/detergent inactivation sample T=120 Minutes	<3.20	>3.57

Module 2: Virus removal by depth filtration

A1HC depth filtration step: Millistak+® Pod Disposable Depth Filter Systems µPod filter – 23 cm^2

- The virus safety evaluation was performed at two different pH conditions: 7.5 (Condition A) and 5.0 (Condition B) of the starting material.
- Virus used for spiking: X-MuLV and MVM
- Process step temperature: 22±2°C
- The "filtrate" was collected and tested after adjusting the pH of the sample to neutral

A1HC depth filtration step

Table 2: A1HC Condition A (pH 7.5) Reduction Factor: X-MuLV

Sample Description	Log_{10} adjusted Viral Titer	Log_{10} Reduction
Load Control	6.88	NA
Processing Control	6.57	NA
Filtrate	<2.78	>4.10

Table 3: A1HC Condition B (pH 5.0) Reduction Factor: X-MuLV

Sample Description	Log_{10} adjusted Viral Titer	Log_{10} Reduction
Load Control	6.68	NA
Processing Control	6.63	NA
Filtrate	<3.48	>3.20

A1HC Depth filtration step

Table 4: A1HC Condition A (pH 7.5) Reduction Factor: MVM

Sample Description	Log_{10} adjusted Viral Titer	Log_{10} Reduction
Load Control	9.61	NA
Processing Control	9.52	NA
Filtrate	2.51	7.10

Table 5: A1HC Condition B(pH 5.0) Reduction Factor: MVM

Sample Description	Log_{10} adjusted Viral Titer	Log_{10} Reduction
Load Control	9.82	NA
Processing Control	9.99	NA
Filtrate	2.51	7.31

Cation Exchange Chromatography (CEX) step

- Tried for mAb product with acidic pI
- Process step temperature: 23±2°C
- The Elution was performed at two different pH conditions 5.5 (Condition A) and 5.0 (Condition B)

Cation Exchange Chromatography (CEX) step

Table 6: CEX Condition A (pH 5.5) Reduction Factor: X-MuLV

Sample Description	Log_{10} adjusted Viral Titer	Log_{10} Reduction
Load Control	6.70	NA
Processing Control	6.48	NA
Eluate	5.12	1.58

Table 7: CEX Condition B (pH 5.0) Reduction Factor: X-MuLV

Sample Description	Log_{10} adjusted Viral Titer	Log_{10} Reduction
Load Control	6.60	NA
Processing Control	6.57	NA
Filtrate	2.49	>4.11

Virus Clearance Summary: Reduction Factor

Table 2: A1HC Condition A (pH 7.5) Reduction Factor: X-MuLV

Process step	Log10 Reduction Factors	
	X-MuLV	MVM
Solvent/detergent inactivation (0.5% Triton X100) T=60 Min	≥ 3.56	-
Solvent/Detergent inactivation (0.5% Triton X100) T=120 Min	≥ 3.56	-
Depth filtration (A1HC) Condition A (pH 7.5)	≥ 4.10	7.10
Depth filtration (A1HC) Condition B (pH 5.0)	≥ 3.20	7.31
CEX- Condition A (pH 5.5)	1.58	-
CEX- Condition B (pH 5.0)	> 4.11	-

Typical process steps followed in Biopharmaceutical industry for mAb's purification and clearance obtained

Process step	Log10 Reduction Factors	
	MuLV	MVM
Affinity Chromatography	2.3	1.9
Low PH inactivation	≥ 5.5	-
AEX Chromatography	≥ 5.2	5.9
Virus retentive filtration (Nanofiltration)	≥ 4.9	≥ 5.8

Summary and Conclusion

- Results showed effective virus clearance for S/D inactivation, Depth filtration and CEX steps.
- Viral clearance from unconventional steps can add value to the total viral clearance independently or along with conventional process steps.
  - Orthogonal strategy for virus clearance along with conventional process steps.
- Helps in building additional capacity for viral clearance into the purification process.

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