Adventitious Viral Agents Control Through Robust rAAV Platform

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Abstract

The ICH released its latest revision of ICH Q5A [R2] guideline on viral safety evaluation of biotechnology products derived from cell lines of human or animal origin in 2023. The revised guideline calls for a risk-based approach to ensuring viral safety of biological products, such as adeno-associated virus (AAV). This presentation will first introduce a full spectrum control strategy highlighting how Oxford Biomedica's transient transfection platform, implemented across multiple AAV serotypes, de-risks endogenous and adventitious virus contamination. This is accomplished through raw material control, single-use closed aseptic processing throughout the platform, dedicated viral intermediate testing, and final drug product characterization. We will then share a rAAV9 viral clearance case study demonstrating robust upstream inactivation kinetics and downstream viral removal performance to deliver significant log reduction value (LRV) clearance throughout the process (Detergent Inactivation->4.67 LRV, Affinity-4.69 LRV, AEX-4.38 LRV), utilizing orthogonal chromatographic modes of separation, namely affinity capture and AEX polishing. We utilized the small-scale model of manufacturing process at current operating parameters, for spiking studies using enveloped Xenotropic murine leukemia virus [XMuLV] and non-enveloped Minute Virus of Mice [MVM] as representative AAV model viruses. We selected MVM due to similar morphology and physicochemical properties as AAV, which may pose competing challenge to be virally cleared from the process. We will showcase that negligible viral particle were detected in the intermediate product pools and no discernible difference in load and elution chromatographic profile was observed, compared to chromatographic profile with no model virus spike [only AAV9]. We will also showcase how this intensified platform is not affected by process feed stream conditions within normal operating range and the data is robust in nature to potentially offer similar LRVs across multiple serotypes, without the need for retentive viral filters (viral filters need process feed adjustments post polishing and pre UFDF steps, impacting product yield).

Finally, we will outline the value proposition offered by Oxford Biomedica's rAAV platform for drug developers in four distinct ways: controls put in place provide barriers to entry and carryover of adventitious agents in the final product, robust viral clearance from orthogonal chromatography unit operations without the need for retentive viral filters, a regulatory IND/BLA package which adheres to the newly released guidance, and the advantage to treat patient populations in a safe and economical manner with negligible immunotoxin profile.

Introduction

The ICH revised guideline calls for risk-based approach to viral safety of biological products, such as adeno-associated virus (AAV). A control strategy should be developed through an assessment of both upstream and downstream manufacturing processing steps.

In this presentation, we will share how the three-pronged control strategy of Oxford Biomedica's multi-serotype rAAV platform de-risks endogenous and adventitious virus contamination through

- ❑ Raw material control and single use closed aseptic processing
- ❑ Dedicated testing of viral intermediates and final drug product characterization
- ❑ A viral clearance study performed using AAV9 feed-stream demonstrating > 4 LRV for XMuLV inactivation through detergent treatment and > 9 LRV for MVM virus clearance through orthogonal chromatography

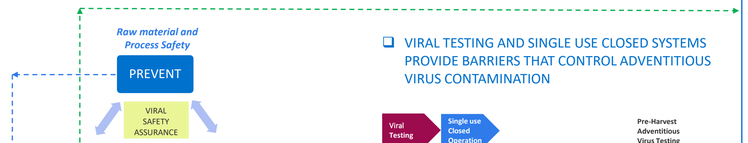
Methods

- Viral clearance studies were performed on manufacturing process small-scale models for unit operations
  - Upstream - Detergent Lysis
  - Downstream - Affinity Capture and AEX Polishing
- AAV9 feed stream material, generated at an Oxford Biomedica facility, was shipped frozen to Texcell Inc. for interference and onsite execution studies.
- Both MVM (parvovirus) and XMuLV (retrovirus) were propagated at Texcell Inc.
- Manufacturing setpoint conditions that were evaluated as the operating parameters for this study:

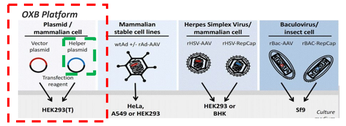
Unit Operation	Pre-Lysis Culture	Affinity Load	AEX Load
Virus Challenge (Log_{10})	8.5 ± 0.5	7.5 ± 0.5	8.5 ± 0.5
Virus	XMuLV (enveloped)	MVM (non enveloped)	MVM (non enveloped)
Method of Viral Clearance	Detergent (Inactivation)	Affinity Resin (Removal by lack of binding, MVM expected in FT*)	AEX Resin (Removal: MVM expected in strip)
Samples Assayed for Viral Clearance	Load, Hold Control, T≤1 minute, T=60 minutes, and T=120 minutes	Load, Hold Control, Affinity pH neutralized Eluate	Load, Hold Control, AEX pH neutralized eluate

*Viral Clearance in a Downstream AAV Process: Case Study Using a Model Virus Panel and a Noninfectious Surrogate. Michael Winkler, Mikhail Goldfarb et alii. Bioprocess International April 2021.

Control Strategy



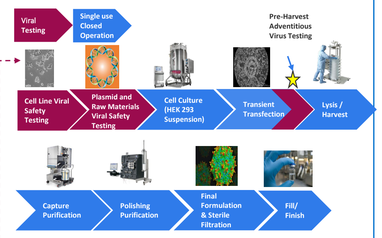
❑ OXB TRANSIENT TRANSFECTION PLATFORM DE-RISKS ENDOGENOUS AND ADVENTITIOUS VIRUS CONTAMINATION THROUGH THE ABSENCE OF HELPER VIRUS



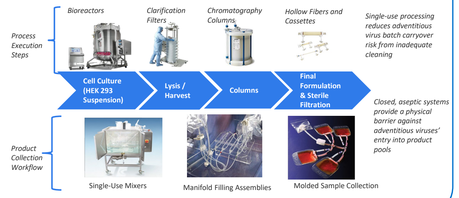
Penaud-Budloo et al., (2018)

- Helper virus systems need robust and dedicated viral inactivation and removal [risk to patient safety]
- Transient transfection platform enhances safety [no viral load]

❑ VIRAL TESTING AND SINGLE USE CLOSED SYSTEMS PROVIDE BARRIERS THAT CONTROL ADVENTITIOUS VIRUS CONTAMINATION



❑ SINGLE USE SYSTEMS PROVIDE PHYSICAL BARRIERS TO ADVENTITIOUS VIRUSES THROUGHOUT THE PROCESS



Viral Clearance Study Results

USP DETERGENT INACTIVATION

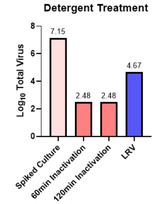


Figure 1: Log total virus and LRV for XMuLV Inactivation by Detergent Treatment at MFG setpoint conditions

- Total virus in load hold control is constant over time
- Post 120 min inactivation, virus titer at assay limit
- >4.67 LRV demonstrates effectiveness of MFG process

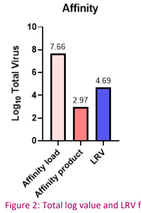


Figure 2: Total log value and LRV for MVM Clearance by Affinity Chromatography at MFG setpoint conditions

- Data shows the ability of Affinity resin to selectively bind AAV
- MVM potentially present in Flowthrough*

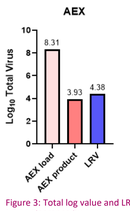


Figure 3: Total log value and LRV for MVM Clearance by AEX Chromatography at MFG setpoint conditions

- No impact on AEX enrichment performance
- MVM potentially present in strip*

DSP CHROMATOGRAPHY CLEARANCE

Table 1: Percentage of spiked MVM virus present in Affinity Product compared to spiked Affinity Load

Sample Description	Total MVM Virus ($TCID_{50}$)	% MVM in fraction compared to Load
Affinity Load	4.57×10^7	N/A
Affinity Product	9.22×10^2	0.00
AEX Load	2.03×10^8	N/A
AEX Product	8.54×10^3	0.00

- No MVM particles detected in Affinity and AEX product

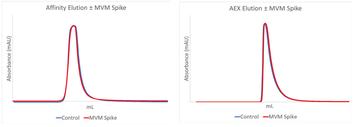


Figure 4: Affinity and AEX elution chromatogram comparison with and w/o MVM Spike

- No difference in profile
- No extra peaks observed
- Elution/strip peaks similar
- UV absorbance ratio similar for blank vs spiked runs

Conclusions

- Overall, > 9 LRV was achieved for MVM virus, and > 4LRV for XMuLV [Table 2]

Table 2: Overall LRV values for XMuLV and MVM across USP and DSP

MFG Steps	XMuLV LRV	MVM LRV
Detergent Inactivation	> 4.67	N/A
Affinity Chromatography	N/A	4.69
AEX Chromatography	N/A	4.38
Overall	> 4.67	9.07

- Risk assessment highlighted the control strategy and value proposition offered by OXB rAAV platform to drug developers in four distinct ways:

Controls provide barriers to entry and carryover of adventitious agents in the final product

Robust viral clearance from orthogonal chromatography unit operations without the need for retentive viral filters

Regulatory IND/BLA package which adheres to the newly released guidance

Advantage to treat patient populations in a safe and economical manner, with negligible immunotoxin profile

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