An Orthogonal Concept for Reliable Virus Clearance in Biomanufacturing

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Abstract:
The virus removal platform technology removes model viruses by three complementary devices: viral retention, Ultra Violet-c (UV) inactivation, and membrane adsorption. The platform is characterized by high overall LRV's for enveloped and non-enveloped viruses. The viral clearance platform offers exceptional additive log reduction values (LRV's) for five relevant model viruses (>12 – 17 LRV). Results demonstrate that the virus inactivation step provides robust elimination of > 5 LRV for all viruses tested with antibody recovery > 95%. Data from the UV inactivation step demonstrates the high clearance potential at three different UV doses and the suitability for the inactivation of small, non-enveloped viruses like Porcine Parvo Virus (PPV) with UV up to 6.5 and a Mab recovery of 97%. In the membrane adsorber step virus removal is depending on the isoelectric point of the virus and therefore a function of the chromatography conditions. Under the process conditions tested effective removal was found for four of the five model viruses. In addition mass balance data from bind & elute studies with Q membranes were generated. A recovery > 98% for Herpes Simplex Virus (HSV) was found demonstrating that removal is clearly depending on adsorption and excluding any effect. An additional study with PRV with a membrane that had no ligands elucidates that membrane adsorbers do not remove virus via sieving effects. The introduced new viral clearance platform is an integrated orthogonal concept for robust and reliable viral clearance.

Introduction:
Recombinant therapeutics manufactured via mammalian cell lines must undergo extensive purification in which viral clearance is addressed. Viral clearance is typically achieved by adding UV's from complementary process components that are part of the purification process. The ICH and EMA have set guidelines for minimal viral clearance values and how they are obtained. At least two supplementary methods must be implemented in order to meet the specifications set forth by these regulatory bodies. Viral retentive filters and virus inactivation have traditionally been accepted technology that removes model viruses regardless of their size and morphology; removal is contingent upon the chemical composition of the mobile phase and the iso-electric point of each model virus species. Sartorius has developed a platform technology that removes model viruses by three complementary devices: viral retention, UV inactivation, and membrane adsorption (MA). The platform is characterized by high virus LRV's and product recovery. Due to their porous nature, MA's possess the greatest potential to compromise the orthogonal nature of this platform technology. Therefore, virus recovery studies were designed to demonstrate that adsorption, not retention is nature of virus removal with MA's.

Results:
Stage I – 20 nm virus retentive filtration:
- Virus retentive nano-filtration, Ultra Violet-c (UV) virus inactivation and Membrane chromatography have been integrated to form a novel orthogonal viral clearance technology platform.
- This newly developed device yields exceptional additive log reduction values (LRV's) for five relevant model viruses (>12 – 17 LRV). Results demonstrate that the virus filtration step provides robust elimination of > 5 LRV for all viruses tested with antibody recovery > 95%.
- Data from the UV inactivation step demonstrates the high clearance potential at three different UV doses and the suitability for the inactivation of small, non-enveloped viruses like Porcine Parvo Virus (PPV) with UV up to 6.5 and a Mab recovery of 97%.

Stage II – UV inactivation:
- In the membrane adsorber step virus removal is depending on the isoelectric point of the virus and therefore a function of the chromatography conditions. Under the process conditions tested effective removal was found for four of the five model viruses. In addition mass balance data from bind & elute studies with Q membranes were generated. A recovery > 98% for Herpes Simplex Virus (HSV) was found demonstrating that removal is clearly depending on adsorption and excluding any effect.

Stage III – Membrane Adsorption:
- An additional study with PRV with a membrane that had no ligands elucidates that membrane adsorbers do not remove virus via sieving effects. The introduced new viral clearance platform is an integrated orthogonal concept for robust and reliable viral clearance.

Validation data from this process demonstrates high overall UV's for enveloped and non-enveloped model viruses.

Conclusions:
- Experiment 2: High Pseudorabies virus LRV with Sartobind Q and negligible LRV with Sartobind base membrane shows that the virus is removed exclusively by adsorption.
- The viral clearance platform offers exceptional additive log reduction values (LRV's) for five relevant model viruses (>12 – 17 LRV).
- Results demonstrate that removal is clearly depending on adsorption and excluding any effect.
- The viral clearance platform offers exceptional additive log reduction values (LRV's) for five relevant model viruses (>12 – 17 LRV).

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