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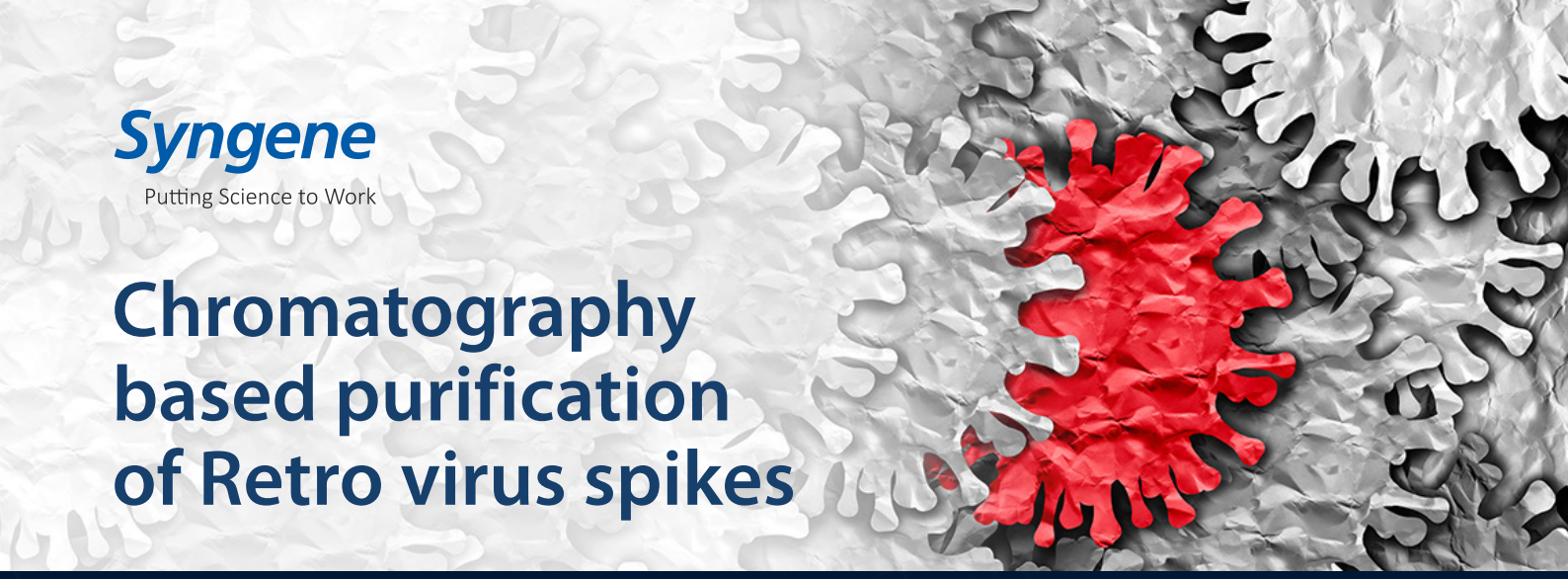
Chromatography based purification of Retro virus spikes



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Abstract

Viral Clearance Study is one among the pillars of safety tripod mentioned in ICH Q5A (R1) guidance document where manufacturing of Antibody should be ensured by demonstrating Viral Clearance on the manufacturing process. Viral Clearance of the Protein purification is demonstrated using relevant model viruses. It is imperative that pure virus spike preparation should be introduced for evaluation of the virus clearance with minimal or no impurities being contributed from the spikes. Keeping this expectation in mind, the virus spike used for the study are purified using various approaches such as ultracentrifugation and chromatography techniques (TR NO 47). The intended goal is to have purified and high tittered virus stock enabled using these techniques. Here, we present the data of purification for Retro virus (RV) using ion exchange chromatography, where the condition for purification was optimized. Loading conditions are pH (7.8), Conductivity (5 ms/cm) Linear flow rate of 50 cm/hr and Elution was optimized to two step gradient for the purified fraction. The chromatography step helps to enhance the purity of virus stock without significantly impacting the infectious titer of the virus. The total protein content in the purified fraction was reduced by 24 fold along with 76% virus recovery. A significant reduction of total contaminant protein ensured pure virus stock was available for virus spiking studies. Thus Ion exchange chromatography provides a robust platform for generating highly purified virus stocks for virus clearance study.

Introduction

- Chromatography is predominant technology used for purification of biomolecules
- Very powerful technique for fractionation of macromolecule rich feedstock, including DNA, proteins and viruses (Lyddiatt, 2002; Morenweiser, 2005).
- Anion exchange chromatography (AEC) is quite stringent, has limiting chromatographic media available.
- AEC has High binding capacity for adsorption and elution of Retroviruses (RV) in non-denaturing conditions, Retroviruses bind strongly to AEC matrices
- The resin needs to separate RVs from contaminants like host cell material and cell culture medium. (Rodrigues T, 2007).
- In present study, AEC conditions were explored to purify the cell culture derived RV stocks intended for virus clearance studies.

Process conditions

For Retrovirus Purification

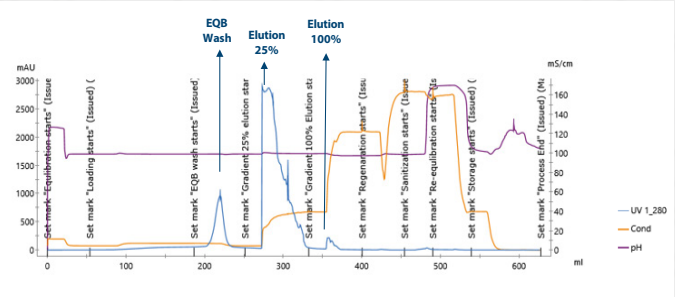
- Anion exchange Resin
- Tris Buffer
- pH (7.8)
- Conductivity (5 ms/cm)
- Linear flow rate of 50 cm/hr
- Elution was optimized to two step gradient (25% and 100%) for purified fraction.
- Desorption at salt concentrations around 1M NaCl.

Methods for evaluation

Following methods were performed to determine the chromatographic performance for virus purity:

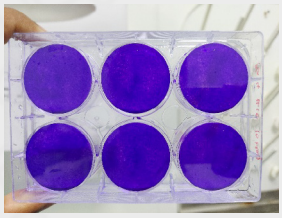
- Spectrophotometry for Protein concentration
- SDS PAGE for Protein Purity determination
- Infectivity Assay for virus titer

Chromatogram for AEC run

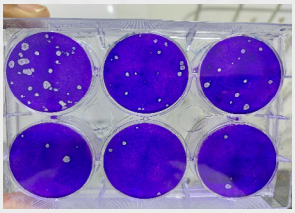


Infectivity Assay on PG-4 Cells

Negative control

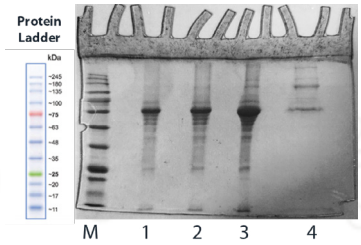


Virus infected plate for titer determination



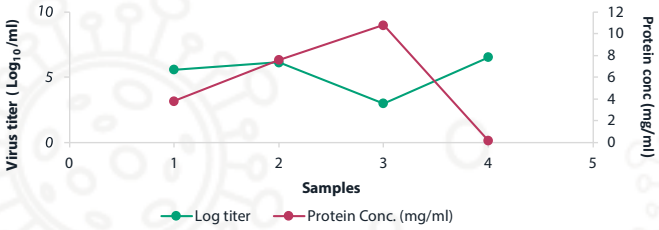
SDS PAGE result

4% stacking gel and 10% resolving gel



M. Marker
1. TFF Load
2. Chromatography Load
3. Elution 25%
4. Elution 100%

Virus Titer (Log_{10} /ml) Vs Protein conc (mg/ml)



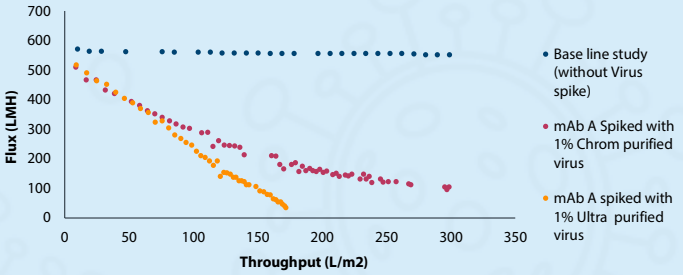
1. TFF Load sample 2. TFF Output/Chromatography load 3. Elution 25% gradient 4. Elution 100% gradient

Result

Sample name	Retro Virus Titer (Log_{10}/ml)	Protein concentration (mg/ml)
Input	5.58	3.82
Output	6.52	0.16

1. Performance of AEC purified virus spike demonstrated low flux decay in the virus filtration studies compared to ultracentrifuged purified virus.

Performance of virus spike preparations



Conclusion

- Total contaminant protein content in purified fraction reduced by 24 fold
- Retro Virus Titer is increased by ~14 fold
- Virus Recovery was 76%
- Contaminant Protein concentration decreases purification of virus increases.
- This method was optimized to Purify the virus for Spiking purpose.
- AEC purified virus spike showed improved performance in viral filtration studies (with Low flux decay) compareed to ultracentrifuged purified virus prep.

References

- Lyddiatt, A., 2002. Process chromatography: current constraints and future options for the adsorptive recovery of bioproducts. Curr.Opin. Biotechnol. 13, 95–103.
- Morenweiser, R., 2005. Downstream processing of viral vectors and vaccines. Gene Ther. 12 (Suppl. 1), S103–S110.
- PDA Technical Report 47: Preparation of Virus Spikes Used for Virus Clearance Studies. Bethesda, MD USA: Parenteral Drug Association,2010.
- Rodrigues T, Carrondo MJ, Alves PM, Cruz PE. Purification of retroviral vectors for clinical application: biological implications and technological challenges. J Biotechnol. 2007 Jan 10;127(3):520-41. doi: 10.1016/j.jbiotec.2006.07.028. Epub 2006 Jul 31. PMID: 16950534.

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