Application Note

ProSep® A Chromatography Resins: Virus Clearance and Sanitization

Introduction

Monoclonal antibodies (mAbs) and other biological drug products carry with them a risk of viral contamination. This risk is generally mitigated through careful screening of source materials and monitoring of process intermediates for the presence of viruses. However, it is possible in rare cases that viruses may enter the process and escape detection. In addition to the possibility of adventitious virus contamination, many cell lines commonly used to produce biologic drugs are known to release endogenous retrovirus-like particles, and these must be sufficiently removed by the drug purification process. It is a regulatory requirement that the drug manufacturing process demonstrates the capability to "clear" or "reduce" viruses[1]. This may be accomplished by either removing virus or by inactivating it so that it is no longer infectious.

Virus clearance is quantified using scaled-down versions of individual drug manufacturing process unit operations. Virus clearance evaluation studies, often referred to as "virus validations," are performed by adding, or "spiking," virus into process intermediates derived from relevant points in the production process. In order to evaluate the robustness of virus clearance, a panel of model viruses representing a range of physiochemical properties are used to challenge the unit operations. The virus clearance contributed by each process step is determined by comparing the amount of virus that enters the operation to the amount that exits and the difference expressed in terms of Log_{10} Reduction Value (LRV), with 1 LRV equaling a 10-fold reduction. A LRV of four or greater is generally considered to be an effective virus clearance step. The LRVs of each applicable process step are then combined to yield an overall LRV for the drug production process.

The Protein A capture step used in many mAb purification processes provides an opportunity for virus clearance. ProSep® A media are protein A affinity chromatography resins generally used as one of the first purification steps in a mAb process. Protein A binds specifically to the constant region of antibodies with high affinity. However, other components of the process material may bind nonspecifically to the purification media, either to the affinity ligand itself or the base matrix. ProSep® A media is manufactured using controlled pore glass (CPG®), which is a rigid, porous open structure that allows rapid mass transfer, unlike agarose based media that is a less open structure and pore diffusion limited. This is illustrated in Figure 1 which shows the complete penetration of IgG into ProSep® Ultra Plus media particles within 5 minutes.

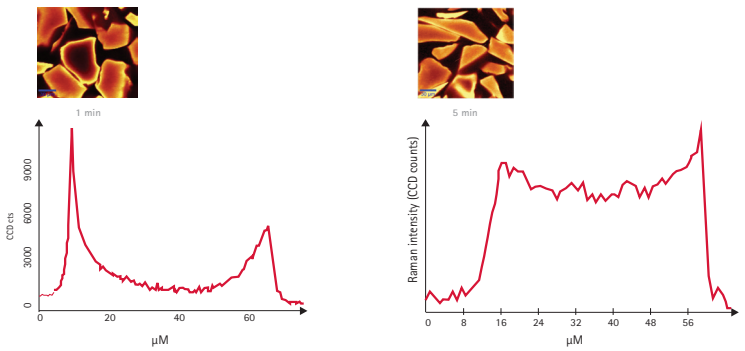

Figure 1.
Confocal Raman microscopy of ProSep® Ultra Plus media. The bright areas on the micrographs show where the IgG has penetrated the ProSep® media. The line scans of the fluorescence intensity correlate to the IgG concentration.

Differences in the structure, materials, and chemistry have the potential to affect the nonspecific binding of viruses to the media.

During the protein A purification step, virus may be separated from the mAb at three points:

- virus may flow through the column when mAb binds;
- virus may be removed from the column during wash steps, and
- virus may remain bound to the column when the mAb is eluted.

Comparison of the amount virus in the feed prior to the Protein A column and virus in the mAb elution provides the LRV for the process.

Studies to evaluate the virus clearance across ProSep® A media demonstrated equivalent or increased virus clearance as compared to an agarose-based protein A media under the conditions tested. The results are presented here together with additional results showing the effectiveness of several ProSep® media sanitization buffers in inactivating viruses.

Virus Clearance Studies

The clearance of several model viruses by ProSep® A media and agarose bead-based protein A media was tested using a selection of diverse model viruses (Table 1). These virus types vary in size and have either an outer lipid envelope or an outer protein shell. This panel represents the range of virus structure and physiochemical resistance typically used for testing biologics. Effective clearance of all viruses in this panel would suggest that a unit operation would be likely to remove an unknown virus as well.

Table 1. Model viruses used for ProSep® media virus clearance studies

Virus/Family	Abbreviation	Model for	Diameter (nm)[1]	Envelope	Genome type	Stability[2]
Minute Virus of Mice/*Parvovirus*	MVM	Adventitious agent	20-26	No	ssDNA	High
Murine Leukemia Virus/*Retrovirus*	MuLV	Endogenous retrovirus-like particles	80-100	Yes	ssRNA	Low
Herpes Simplex Virus Type 1/*Herpesvirus*	HSV	Adventitious agent	120-200	Yes	dsDNA	Low
Poliovirus Type 2/*Picornavirus*	Polio	Adventitious agent	28-30	No	ssRNA	High

1 As cited in Technical Report No. 47: Preparation of Virus Spikes Used for Virus Clearance Studies. Parenteral Drug Association. 2010.
2 Stability reflects the ability of the virus to persist in the environment and resist inactivation by chemical means, such as low pH or detergent treatment.

Two independent studies were conducted to evaluate the virus clearance provided by several types of ProSep® A media. Agarose bead-based protein A media was also tested for comparison. Protein A media was packed into 7cm Omnifit columns (volume 2.4 mL) or similar columns. Clarified cell culture supernatants were spiked with virus and applied to protein A media columns. Antibody from the supernatants was purified in a typical bind-wash-elute purification cycle, using the buffers detailed in Table 2.

Table 2. Buffers used for protein A clearance studies

Viruses Tested	MVM	MuLV, HSV, and Polio
Feed material	Clarified CHO culture supernatant from mAb-producing clone – total protein content 4mg/mL (0.22µm filtered)	Clarified cell culture supernatant (0.45µm filtered)
Column equilibration buffer	Phosphate buffered saline (PBS)	Phosphate buffered saline (PBS)
Binding/wash buffer	0.1M citrate pH 5.0 (0.22µm filtered)	1 M glycine/0.15M NaCl, pH 8.6 (0.45µm filtered)
Elution buffer	0.1M citrate pH 3.5 (0.22µm filtered)	0.1M citrate pH 5.0 (0.45µm filtered)

Table 3. Summary of virus clearance by ProSep® A media and agarose bead-based protein A media

Media	MVM	MuLV	HSV	Polio
ProSep® Ultra Plus	4.4			
ProSep® Ultra	4.3			
ProSep®-vA High Capacity	4.1	6.4	≥7.1	5.9
Agarose-based protein A media	3.2	6.8	4.4	4.5

*blank cell indicates not tested

Virus clearance by the various types of CPG®-based ProSep® A media was equivalent to or greater than the clearance achieved by agarose bead-based Protein A media for all viruses tested; differences of less than ± 0.5 log are not considered significant due to the inherent error in the virus titration assays.

ProSep® Media Sanitization

A study was performed to define the kinetics of virus inactivation by various ProSep® A media sanitization solutions. Two model viruses were used in this study: xenotropic murine leukemia virus (X-MuLV), an enveloped virus, and minute virus of mice (MVM), an un-enveloped parvovirus (Table 4). Viruses with an outer lipid envelope are more susceptible to chemical inactivation than un-enveloped viruses.

ProSep® media is compatible with a number of sanitization buffers, including acids and alcohol. Several ProSep® media sanitization buffers were spiked with X-MuLV and MVM to quantify the inactivation of the viruses over time (Table 4). The buffers were spiked with either X-MuLV to a titer of 6.3 log TCID50/mL or MVM to a titer of 7.6 log TCID50/mL. Samples were titrated for virus at the time points indicated. Appropriate dilution of samples was performed to avoid cytotoxicity in the cell-based virus infectivity titration assays. The log reduction values for the viruses under the various conditions are shown in Tables 5 and 6, with a summary in Table 7.

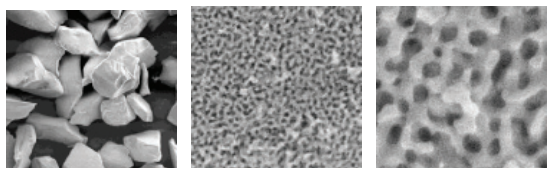

Figure 2.
Electron Micrographs showing the porous structure of CPG®

Table 4. Buffers used for ProSep® media sanitization

Buffer composition (all made in Milli-Q® purified water)	Times tested
100mM Phosphoric Acid	1, 10, 20, 60 min
150mM Phosphoric Acid	1, 10, 20, 60 min
PAB: 167mM Sodium Acetate Buffer, 120mM Phosphoric Acid; 2.2% Benzyl Alcohol	1, 10, 20, 60 min
1% Benzyl Alcohol; 100mM Sodium Acetate Buffer, pH 5.2	1, 4, 8, 24 hr
2% Benzyl Alcohol; 100mM Sodium Acetate Buffer; pH 5.0	1, 4, 8, 24 hr
TNE (control buffer): 10 mM Tris, 100 mM NaCl, 1mM EDTA	1 min; 1, 4, 8, 24 hr

Table 5. X-MuLV (retrovirus) log reduction values (LRV)

	100 mM phos acid	150 mM phos acid	PAB	1% benzyl alcohol	2% benzyl alcohol	TNE
pH	1.5	1.3	2.6	5.2	5.0	7.4
1 min	≥ 5.1[1]	≥ 5.1	≥ 4.6			0.0
10 min	≥ 5.1	≥ 5.1	≥ 4.6			
20 min	≥ 5.1	≥ 5.1	≥ 4.6			
60 min	≥ 5.1	≥ 5.1	≥ 4.6	1.9	≥ 4.6	-0.1
4 hrs				3.7	≥ 4.9	-0.1
8 hrs				≥ 5.4	≥ 5.4	-0.6
24 hrs				≥ 4.8	≥ 4.8	0.1

[1] An LRV listed as "≥ X" indicates that no virus was detected in that sample. The LRV given was calculated based upon the virus titer in the corresponding control sample and the amount of fluid screened for the presence of virus.

Table 6. MVM (parvovirus) log reduction values (LRV)

	100 mM phos acid	150 mM phos acid	PAB	1% benzyl alcohol	2% benzyl alcohol	TNE
pH	1.5	1.3	2.6	5.2	5.0	7.4
1 min	0.2	0.1	0.8			0.0
10 min	0.3	0.1	0.5			
20 min	0.6	0.2	0.7			
60 min	1.0	1.1	0.6	0.2	0.5	0.0
4 hrs				0.4	0.4	-0.1
8 hrs				0.1	0.3	0.1
24 hrs				0.1	0.9	0.3

*blank cell indicates not tested

Table 7. Summary of sanitization buffers LRVs at latest time points tested

Buffer	X-MuLV		MVM	
	LRV	Effective[3]	LRV	Effective[3]
100 mM phosphoric acid	≥ 5.1	Yes	1.0	No
150 mM phosphoric acid	≥ 5.1	Yes	1.1	No
PAB[1]	≥ 4.6	Yes	0.6	No
1% Benzyl Alcohol[2]	≥ 5.4	Yes	0.4	No
2% Benzyl Alcohol[2]	≥ 5.4	Yes	0.9	No

[1]PAB: 167 mM sodium acetate buffer, 120 mM phosphoric acid, 2.2% benzyl alcohol
[2]100 mM sodium acetate with 1 or 2% benzyl alcohol
[3]Effective - defined as reduction of at least 4 logs

The acidic buffers (100mM and 150mM phosphoric acid and PAB) were effective at inactivating X-MuLV. After one minute, X-MuLV was reduced to non-detectable levels. As expected, these buffers were much less potent against MVM, and inactivation was time dependent, reaching a LRV of approximately 1 after one hour (the latest time point tested).

X-MuLV inactivation by benzyl alcohol was time and concentration dependent. 1% benzyl alcohol required 8 hours to reduce virus to non-detectable levels, while 2% benzyl alcohol was maximally effective by 1 hour (the first sampled time point). MVM was resistant to benzyl alcohol buffers, reaching a LRV of 0 to 1 after 24 hours.

Conclusions

The results of these studies demonstrate that ProSep® A chromatography media can effectively contribute to the viral clearance of the mAb purification process. Furthermore, common sanitization buffers compatible with ProSep® A media are effective against retrovirus but not against a model parvovirus.

References

1 ICH Harmonized Tripartite Guideline: Q5A Viral Safety of Biotechnology Products Derived from Cell Lines of Human or Animal Origin. Fed. Reg. 63(185) 24 September 1998: 51074.

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