APPLICATION NOTE

GORE® Protein Capture Devices: Increased Productivity at Multiple Scales with Rapid CIP

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Objective

This application note demonstrates utilizing a rapid CIP and optimizing the loading and non-loading residence times during cycling to achieve increased productivity on a Gore Protein Capture Device. The method is easily transferred from lab scale to pilot scale sizes as demonstrated using both PROA101 (1mL) and PROA201 (58 mL) Gore Protein Capture Devices.

Purpose

Cleaning during the Protein A step in antibody purification cycling is crucial as bioburden and endotoxin could be introduced to the system from the harvest feed stream or an external source, creating a risk of contamination. Resin chromatography columns typically employ a 3-15 minute contact time using 0.1 to 0.5 M NaOH during cycling.

Due to Gore Protein Capture Devices (GPCD) employing a unique ePTFE membrane composite that is a somewhat less hydrophilic stationary phase compared to a highly hydrophilic resin, such as agarose gel, it is hypothesized that the aforementioned cleaning method may be overly conservative to mitigate bioburden and endotoxin during cycling. A high flow rate convective cleaning step with a high molarity NaOH buffer and less contact time may be sufficient to maintain acceptable bioburden and endotoxin levels in eluates through a higher number of cycles. The proposed method would be faster and consume less NaOH than the incumbent method. It would also increase productivity by eliminating ~3 minutes of cycle time per cycle.

Cycle time can also be shortened by reducing both loading and non-loading step times. Residence times faster than 20 SRT and 10 SRT for loading and non-loading steps, respectively, are of interest in order to achieve increased productivity.

Materials and Equipment

- AKTA Pilot 600S Liquid Chromatography System
- AKTA Pure 150 Liquid Chromatography System
- Trastuzumab Biosimilar Clarified CHO Cell Harvest with 3.96 g/L titer
- 1.0 mL Gore Protein Capture Device (PROA101)
- 58.0 mL Gore Protein Capture Device (PROA201)
- Chemicals outlined in protocols on the tables

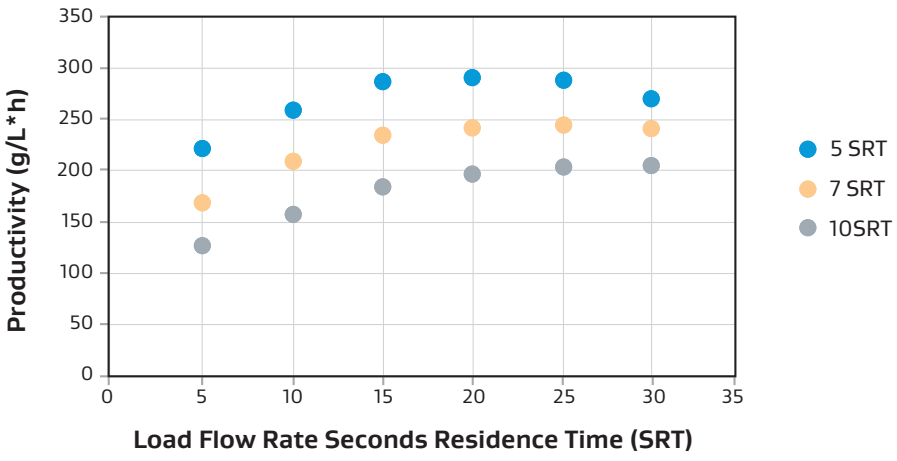
Procedure

The increased productivity flow rates were chosen by running several 58 mL scenarios shown in Table 1 through a productivity calculator, similar to Productivity Calculator - GORE® Protein Capture Device with Protein A and was modified to assess faster residence times, using a 30L bioreactor with 4 g/L titer as the inputs. The $DBC_{10\%}$ values in Table 1 are based on the product specification and polyclonal human IgG as the target molecule. The actual $DBC_{10\%}$ values differ based on the molecule used.

Table 1: Theoretical Productivity Calculations at differing input conditions for a 58mL Device (PROA201)

Load Residence Time in seconds (SRT)	Predicted DBC10% (mg/mL)	Non-Load Residence Time in seconds (SRT)	Cycle Time (min)	Number of Cycles Needed to clear 30 L reactor	Time to clear reactor (h)	Buffer Consumption (L)	Productivity (g/L*h)
30	40	10	9.33	65	10.11	121	205
		7	7.88		8.54		242
		5	6.92		7.49		276
25	35	10	8.25	74	10.18	138	203
		7	6.80		8.39		247
		5	5.83		7.19		288
20	30	10	7.33	87	10.63	162	195
		7	5.88		8.53		243
		5	4.92		7.13		290
15	25	10	6.58	104	11.41	194	181
		7	5.13		8.90		233
		5	4.17		7.22		286
10	20	10	6.00	130	13.00	242	159
		7	4.55		9.86		210
		5	3.58		7.76		266
5	15	10	5.62	173	16.21	322	128
		7	4.17		12.03		172
		5	3.21		9.25		224

Figure 1: Theoretical PROA201 Productivity at 3 non-loading flow rates over a range of loading flow rates



Tables 2 and 3 outline the 1.0 mL (PROA101) and 58 mL (PROA201) protocol used per cycle, respectively. Although 5 SRT non-load would be the most optimal productivity based on Figure 1, 7 SRT was chosen for non-loading steps to be conservative with regard to the recommended maximum operating pressure drop of ≤0.4MPa.

15 SRT was chosen as the load residence time based on Figure 1 and aligned with the purpose of reducing cycle time.

Table 2: 1.0 mL Device (PROA101) Cycling Protocol

	Step	Buffer	Column Volumes (CV)	Residence Time in seconds (SRT)	Flow Rate (mL/min)	Pump Wash
1	Equilibration	TBS*	5	7	8.57	No
2	Load	Trastuzumab	Load to 80% of 10% BT	15	4	No
3	HS Wash	HS TBS (1.15M NaCl)	4	7	8.57	Yes
4	Equilibration	TBS	5	7	8.57	Yes
5	Elution	Acetic Acid Buffer**	6	7	8.57	Yes
6	Acid Strip	100mM Citric Acid pH 2	4	7	8.57	Yes
7	CIP	0.5M NaOH	3	10	6	Yes
8	Equilibration	TBS	5	7	8.57	Yes

* 50mM Tris, 150mM NaCl pH 7.4 **100mM Acetic Acid pH 3

Table 3: 58 mL Device (PROA201) Cycling Protocol

	Step	Buffer	Column Volumes (CV)	Residence Time in seconds (SRT)	Flow Rate (mL/min)	Pump Wash
1	Equilibration	TBS*	5	7	497.1	No
2	Load	Trastuzumab	Load to 80% of 10% BT	15	232	No
3	HS Wash	HS TBS (1.15M NaCl)	4	7	497.1	No
4	Equilibration	TBS	5	7	497.1	No
5	Elution	Acetic Acid Buffer**	6	7	497.1	Yes
6	Acid Strip	100mM Citric Acid pH 2	4	7	497.1	No
7	CIP	0.5M NaOH	3	10	348	Yes
8	Equilibration	TBS	5	7	497.1	Yes

* 50mM Tris, 150mM NaCl pH 7.4 **100mM Acetic Acid pH 3

ELISAs for Purity

The amount of Protein A leached from the devices was evaluated using a Protein A ELISA kit from Repligen Bioprocessing, P/N 9000-1 (Repligen Corporation, 41 Seyon Street, Building 1 Suite 100, Waltham, MA 02453). The ELISA was performed using the "Dilute and Go" extraction according to the manufacturer's instructions.

Host Cell Protein (HCP) concentration in elution pools obtained during purification cycles was determined using Cygnus kit #F550-1 (Cygnus Technologies, Southport, NC 28461) following manufacturer protocols.

Elution Width

Elution widths were determined from chromatograms using 100 mAU - 100 mAU as the cutoff.

Yield

Yield was determined from concentration of the mAb in the elution fractions, which was calculated by measuring the absorption at a wavelength of 280 nm using a Little Lunatic UV/Vis Spectrophotometer (Unchained Labs, 6870 Koll Center Parkway, Pleasanton, CA 94566) and an extinction coefficient of the IgG1 of 1.47 mL g^{-1} cm^{-1}.

Pressure

Column pressure drop was determined from the chromatograms at the Equilibration, Load, and Elution steps.

Endotoxin

Endotoxin was determined from a kinetic-turbidimetric LAL assay of the elution fractions following standards established in USP <85>, USP <161>, and ANSI/AAMI ST72.

Bioburden

Bioburden was determined from analysis of elution fractions following guidance from USP <1231>.

Results

Cycling Using Increased Productivity Protocol

The protocol detailed in Tables 2 and 3 was used to perform 100 cycles on a 1.0 mL (PROA101) device and 10 cycles on a 58 mL (PROA201) device. Figure 2 shows the overlayed chromatograms of selected PROA101 and PROA201 cycling runs. The pressure drop and elution width product specifications are ≤0.4 MPa, and ≤3.5 CVs when assessed 100 mAU-100 mAU, respectively. Both remained well below product specifications during cycling, as shown in Figures 3 and 4.

The devices were loaded to 80% of the Dynamic Binding Capacity (DBC) at 10% breakthrough. Prior to cycling, DBC at 10% breakthrough at 15 SRT loading residence time was determined for each device using purified Trastuzumab biosimilar (3.1 g/L) to determine the load amount.

Figure 2: Left: 1.0 mL cycles 1, 25, 50, 75, and 100 overlayed; Right: 58 mL cycles 1, 5, and 10 overlayed.

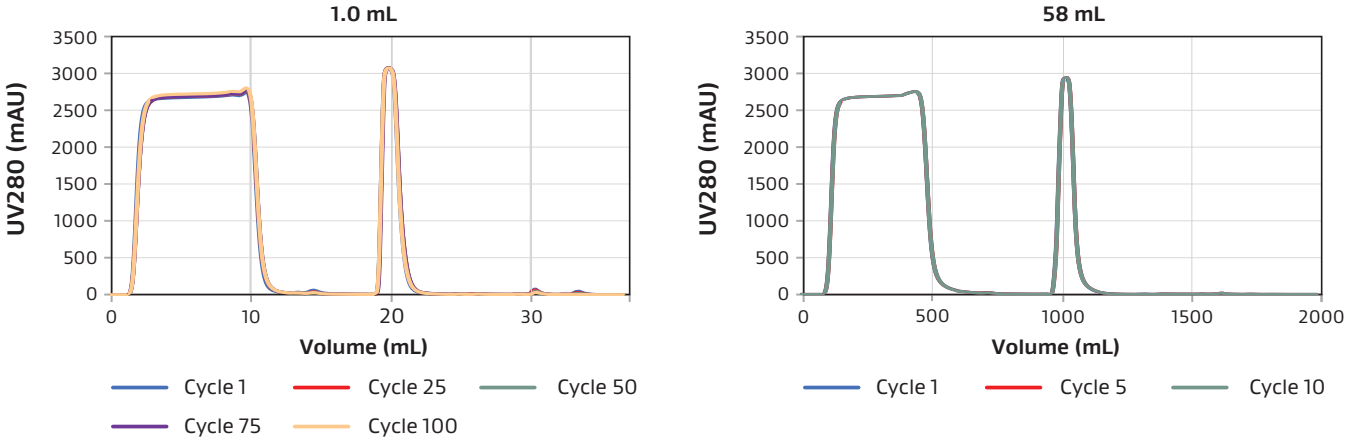


Figure 3: Left: 1.0 mL Pressure Drop data with red line indicating acceptance criterion of ≤0.4 MPa; Right: 58 mL Pressure Drop data with red line indicating acceptance criterion of ≤ 0.4 MPa

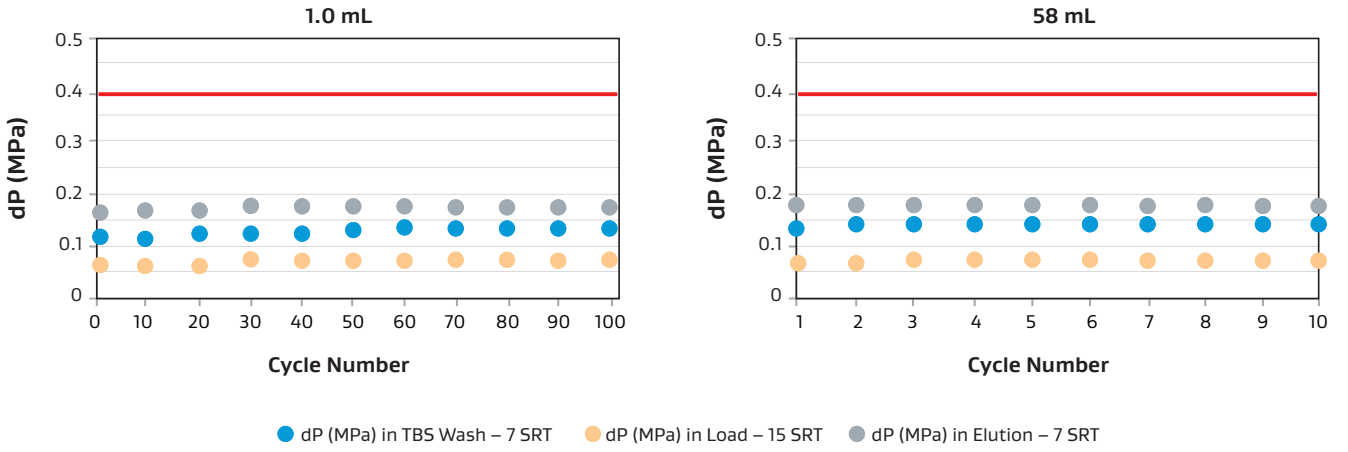
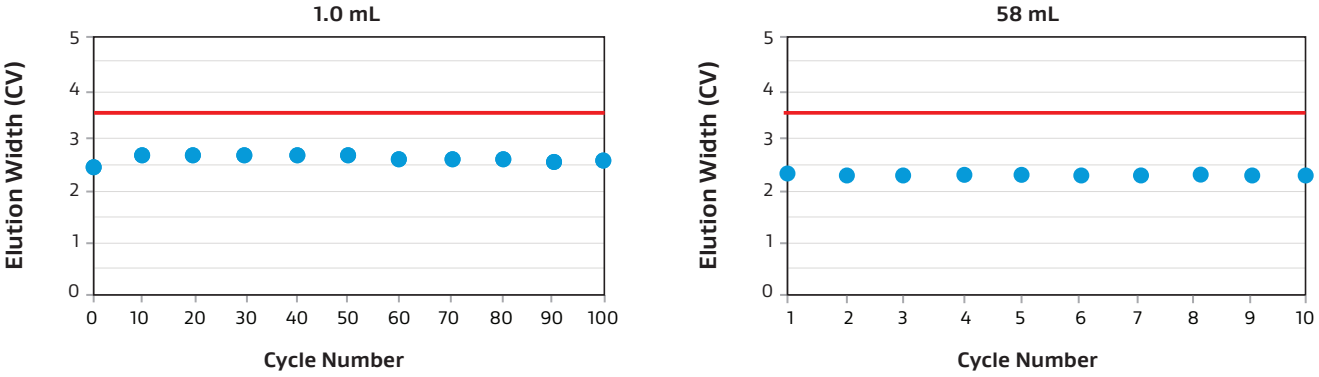


Figure 4: Left: 1.0 mL Elution Width data in column volumes over 100 cycles with red line indicating acceptance criterion of ≤3.5 CV; Right: 58 mL Elution Width data in column volumes over 10 cycles with red line indicating acceptance criterion of ≤3.5 CV



Achieving Increased Productivity

The eluate from cycles during the beginning, middle, and end of 100 cycles on the 1mL were pooled together for endotoxin and bioburden testing purposes, and the yield, leached protein A, host cell protein clearance, and productivity data were extracted from those pooled cycles. These results can be found in Tables 5 and 7. The cycle time for the protocol outlined in Table 2 was 8.88 minutes, which enabled an average productivity of 212 g/L*h for the 1.0 mL over 100 cycles. A total of 33 mg of protein was loaded per cycle.

Elution fractions from the beginning, middle, and end of the 58 mL device run were tested for endotoxin and bioburden as well as yield, leached protein A, host cell protein clearance, and productivity. These results can be found in Tables 6 and 7. The cycle time for the protocol outlined in Table 3 was 6.88 minutes, which enabled an average productivity of 207 g/L*h. A total of 1414.94 mg of protein was loaded per cycle.

For both device sizes, the yields were >90% over the course of cycling, and the productivity was consistent and high. The protein A leaching performance met the acceptance criterion of ≤ 20 ppm. As shown in Tables 6 and 7, all values were below 1 ppm and some were below the detection limit (BDL).

Table 5: 1.0 mL Cycling Data

Pooled Cycles	Cycles 1-6	Cycles 47-52	Cycles 95-100
Yield (%)	95%	95%	95%
Productivity (g/L*h)	213	213	212
Leached Protein A (ppm)	0.98	0.57	0.65

Table 6: 58 mL Cycling Data

Pooled Cycles	Cycle 1	Cycle 5	Cycle 10
Yield (%)	97%	97%	97%
Productivity (g/L*h)	206	207	207
Leached Protein A (ppm)	0.78	BDL*	BDL*

*Below Detection Limit of 0.50 ppm

Figure 5: Left: 1.0 mL yield over 100 cycles with red line indicating acceptance criterion of ≥90%; Right: 58 mL yield over 10 cycles with red line indicating acceptance criterion of ≥90%

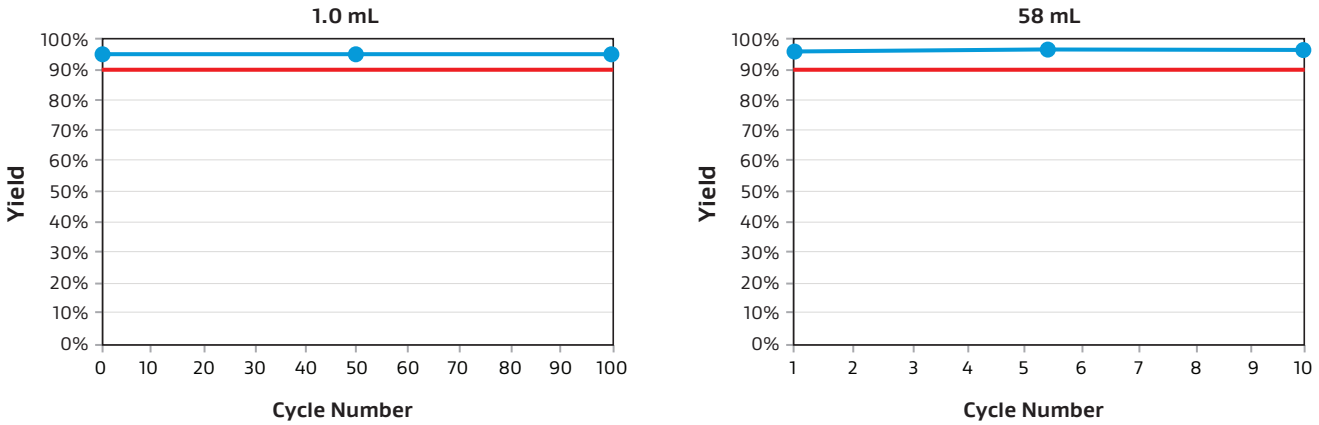


Figure 6: Left: 1.0 mL Productivity over 100 cycles; Right: 58 mL Productivity over 10 cycles

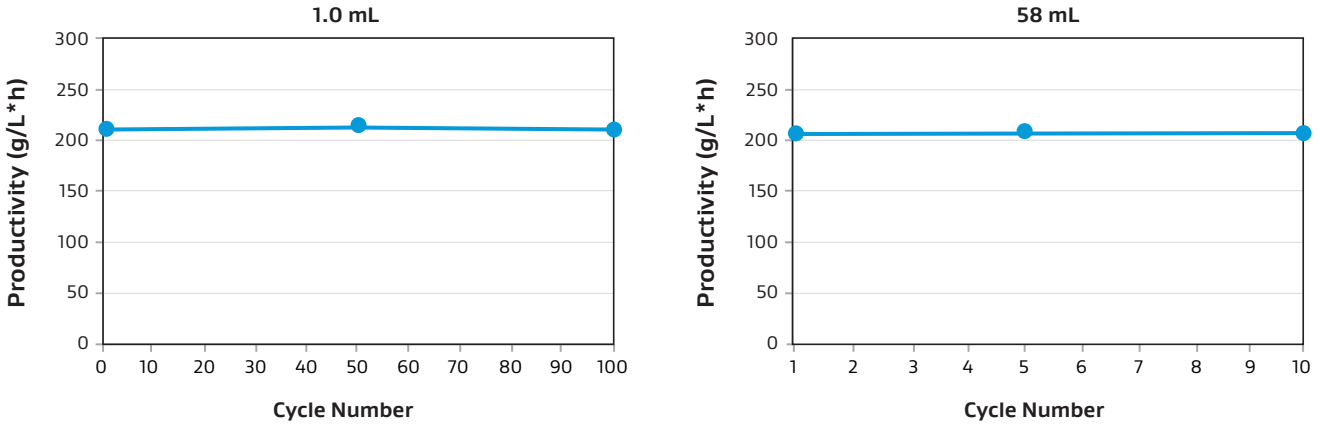
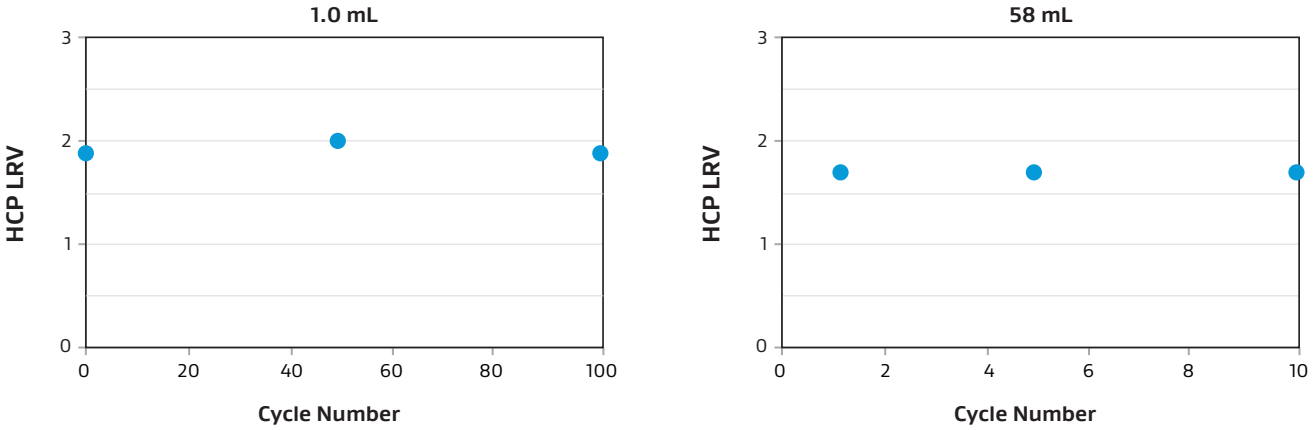


Table 7: 1.0 mL and 58 mL HCP Cycling Data

Device Size	Cycle Number	HCP in elution pool (ng/mg)	HCP LRV	HCP in CHO cell harvest (ng/mg)
1 mL	1-6	756.2	1.90	60665
	47-52	636.9	1.98	
	95-100	794.2	1.88	
58 mL	1	1403.9	1.72	73065
	5	1450.6	1.70	
	10	1566.1	1.67	

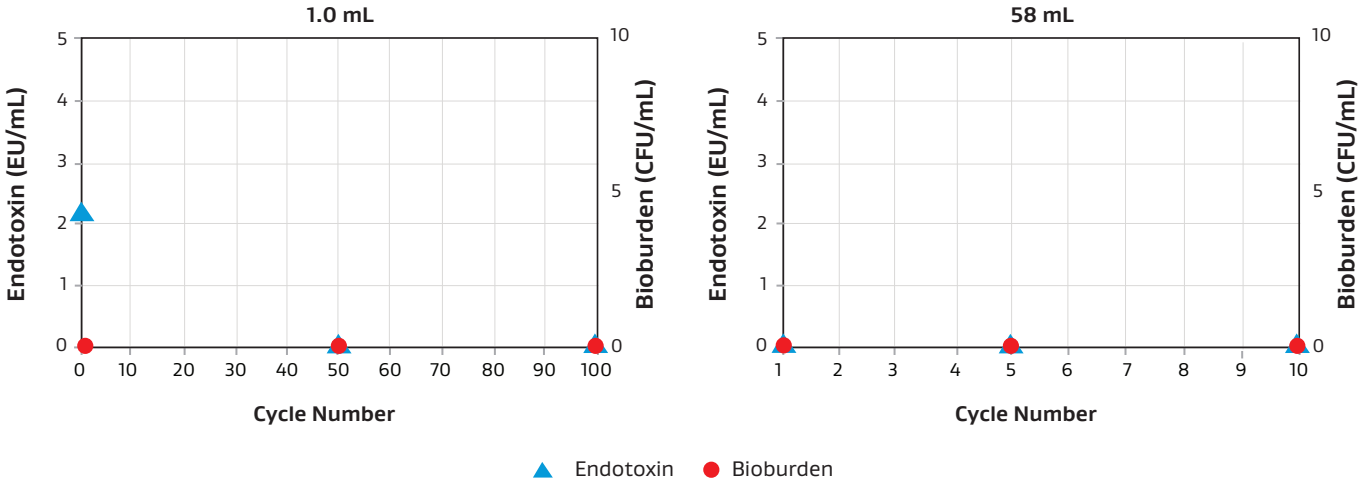
Figure 7: Left: 1.0 mL HCP clearance data over 100 cycles; Right: 58 mL HCP clearance data over 10 cycles



Effectiveness of Rapid CIP

All endotoxin and bioburden levels in elution pools during cycling were within the acceptance criteria limits of <10 CFU/mL and <5 EU/mL. The 58 mL endotoxin results were all <0.125 EU/mL, which are represented as 0 EU/mL in Figure 8. The 58 mL bioburden results were all 0 CFU/mL. The 1.0 mL endotoxin results were 2.217 EU/mL for the initial pooled cycles 1-6, and the rest of the pooled cycles were <0.1EU/mL, which are represented as 0 EU/mL in Figure 8. The 1.0 mL bioburden results were all 0 CFU/mL.

Figure 8: Left: 1.0 mL Endotoxin and Bioburden cycling data; Right: 58 mL Endotoxin and Bioburden Cycling Data



Conclusions

The rapid CIP method of 30 second 0.5M NaOH contact time is an effective way to perform a CIP on GORE Protein Capture Devices. The 1.0 mL device was not sanitized prior to the start of cycling. The cycling procedure was able to clear the small amount of endotoxin that was present at the start of the device run. Both the PROA101 and PROA201 devices met the endotoxin and bioburden acceptance criteria, with 0 CFU/mL present in their elution pools over the course of cycling, and endotoxin <0.1 EU/mL for the majority of cycling. This confirms that the rapid CIP procedure is sufficient to maintain acceptable bioburden and endotoxin levels while cycling.

The pressure drop across both device sizes was <0.2 MPa while cycling at the increased flow rates. The elution widths were consistent and <3.5 CVs throughout cycling for both the 1.0 mL and 58 mL. The yields for both device sizes were above 90% and did not decline during cycling even with increased concentration of base for the CIP. The protein A leaching values were <1 ppm for all cycles on both devices, and the HCP clearance was between 1.7 – 2 LRV with a harvest with a low starting HCP concentration. Both the 1.0 mL and 58 mL devices exceeded the criteria established for the product critical quality attributes, while achieving productivities of 212 and 207 g/L*h, respectively. The results in this Application Note demonstrate that the productivity of a GORE Protein Capture Device can be further increased by decreasing loading residence time to 15 SRT and decreasing non loading residence time to 7 SRT while maintaining performance in other critical quality attributes of the product when run at less productive conditions.

Gore PharmBIO Products

Our technologies, capabilities, and competencies in fluoropolymer science are focused on satisfying the evolving product, regulatory, and quality needs of pharmaceutical and bioprocessing customers, and medical device manufacturers. GORE® Protein Capture Devices with Protein A, like all products in the Gore PharmBIO Products portfolio, are tested and manufactured under stringent quality systems. These high-performance products provide creative solutions to our customers' design, manufacturing, and performance-in-use needs

NOT INTENDED FOR USE in medical device or food contact applications or with radiation sterilization. GORE Protein Capture Devices are intended for research use only and should not be used for clinical or diagnostic procedures.

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