Use of Capto ViralQ for the removal of genomic DNA from influenza virus produced in MDCK cells

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Application note 28-9769-69 AB Vaccines

Use of Capto™ ViralQ for the removal of genomic DNA from influenza virus produced in MDCK cells

We have developed a chromatographic method to remove host cell derived genomic DNA (gDNA) from cell-cultured influenza virus. Capto ViralQ medium (resin) was used to scavenge gDNA while keeping the virus in the flowthrough. The process encompassed screening of optimal running conditions in 96-well filter plates filled with Capto ViralQ to a complete method in a 20 mL column; including gDNA binding capacity determination. The results show that using a buffer with 500 mM NaCl was appropriate for all four different influenza strains that were tested. Viral yields were in the range of 70% to 85% and gDNA removal was over 99.9% with a log-reducing factor of 2 to 4—depending on the amount of gDNA in the starting sample.

Introduction

One of the major contaminants that has to be removed in the course of purifying influenza virus for the production of vaccines is genomic/chromosomal gDNA (gDNA). In this application note, we describe a chromatographic step for removing gDNA from a virus-containing feed. The entire method—from screening of running conditions to final method—was performed at laboratory scale. Capto ViralQ medium was used to scavenge gDNA and leave the virus in the flowthrough. Quantitative PCR (qPCR) was used to determine gDNA concentration and single radial immunodiffusion (SRID) was used to determine the amount of Hemaglutinin (HA) as a measure of virus concentration.

Materials and methods

Cell culture and Infection

The influenza viruses were cultivated in Madine-Darby Canine Kidney (MDCK) cells grown on Cytodex™ microcarriers. The feed was harvested 4 d after infection.

Clarification

Clarification was performed by pumping the harvest through two ULTA™ GF capsules in series starting with 2.0 µm followed by 0.6 µm.

Concentration/diafiltration

The concentration step was performed with a 650 cm^2 hollow fiber with NMWC of 500 kDa. The clarified feed was first concentrated 10 times followed by a 6-fold diafiltration into 20 mM Tris-HCl, 0.5 M NaCl, pH 7.5, after which an additional 2-fold concentration was performed. Thus in total, the feed was concentrated 20 times. Three seasonal influenza strains, A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 were run with this procedure with similar results. In each case, about 80% of the total protein was removed.

Screening for optimal pH and ionic strength

A Design of Experiments (DoE) approach was used to screen pH and ionic strength of the buffer for optimum gDNA removal and maximum virus (HA) recovery. The material was a clarified and concentrated feed of A/Solomon Islands/3/2006 (H1N1). Screening was performed with 96-well filter plates filled with Capto ViralQ using 20 mM Tris-HCl with a NaCl concentration of 300 to 700 mM and a pH range of 7 to 8.

The results from the screening experiment showed that the amount of gDNA in the flowthrough increased with increasing ionic strength (i.e., towards the red portion of the contour plot in Figure 1). The amount of gDNA in the flowthrough was unaffected by pH and no significant change in HA recovery was found with the sodium chloride concentrations. We chose an optimal pH of 7.5 and a salt concentration of 500 mM NaCl.

The results from the screening experiments were verified with each virus strain on a column packed with Capto Viral Q using buffers containing salt concentrations of 400, 500, and 600 mM NaCl. The chromatography results were similar for all the virus strains.

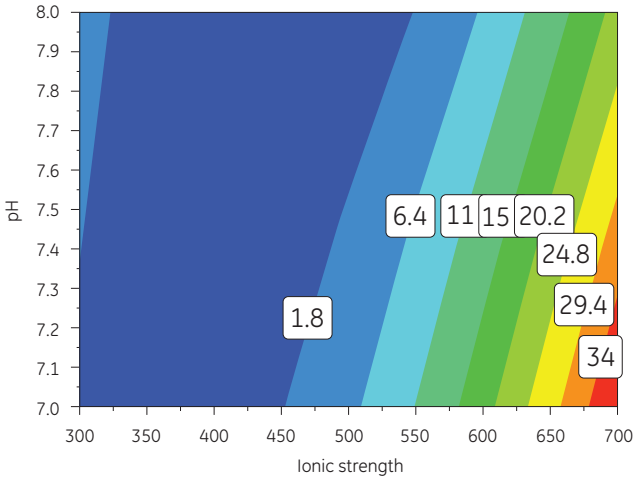

Fig 1. Screening of optimal chromatographic conditions with Capto ViralQ in 96-well filter plates. The plot displays the dependence of gDNA recovery in the flow through of pH and concentration of NaCl in the running buffer. The relative level of gDNA is shown in white boxes in the plot. Optimal conditions with the lowest attainable level of gDNA present in the flowthrough exist in the dark blue region.

Determination of gDNA binding capacity

A 1 mL HiTrap™ column was used to determine gDNA binding capacity of Capto ViralQ. Prior to the run, the sample (~ 17 µg gDNA/mL) was clarified and concentrated as described above. The sample load was 15 mL and 2 mL fractions were collected and analyzed via qPCR.

The chromatogram in Figure 2 shows the UV curve at 280 nm (in blue) and the gDNA content of the fractions is displayed in the histogram (red). Approximately 20% of the total amount of the gDNA loaded was recovered in the elution peak while the remaining 80% was eluted after CIP.

Column: Capto ViralQ 1 mL HiTrap column
Buffer A: 20 mM Tris, 0.5 M NaCl, pH 7.5
Buffer B: 20 mM Tris, 1.5 M NaCl, pH 7.5
Sample: 15 CV concentrated Influenza A/PR/8/34 (H1N1), produced in MDCK cells
Flow: 150 cm/h

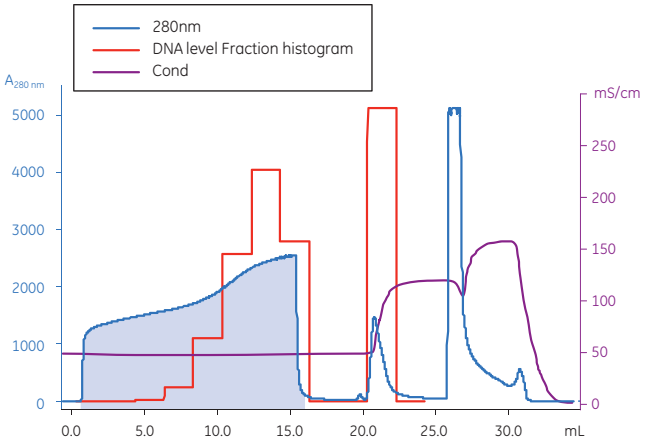

Fig 2. Chromatogram showing the UV curve at 280 nm (blue) and the amount of gDNA detected in the fractions, represented as histograms and shown in red. Conductivity is shown in lilac.

Analysis of the first three fractions of the flowthrough showed no presence of gDNA. However, in the fourth fraction about 1% of gDNA was recovered (Fig 3). The gDNA binding capacity was about 100 µg/mL, which corresponds to 6 CV.

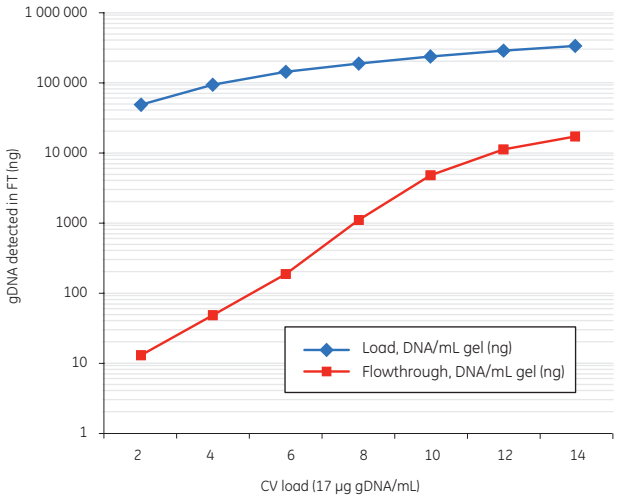

Fig 3. Diagram showing the binding capacity of Capto ViralQ for DNA, the number of column volumes (1 CV = 1 mL) loaded, and the amount of gDNA detected in the flowthrough.

Life cycle study

Figure 4 shows 10 runs of a life-cycle study involving loading, elution, and cleaning-in-place (CIP). The load was 2.5 CV of sample A/PR/8/34 (H1N1). Elution was performed with increasing steps of NaCl concentrations. The column was cleaned with NaOH between runs. SRID was used to measure the amount of virus present and qPCR was used to determine gDNA concentration in the flowthrough and elution peaks from the first, fifth, and tenth cycles.

Column: Capto ViralQ 1 mL HiTrap column
Buffer A: 20 mM Tris, 0.5 M NaCl, pH 7.5
Buffer B: 20 mM Tris, 1.5 M NaCl, pH 7.5
Sample: 2.5 CV concentrated Influenza A/PR/8/34 (H1N1), produced in MDCK cells
Flow: 150 cm/h

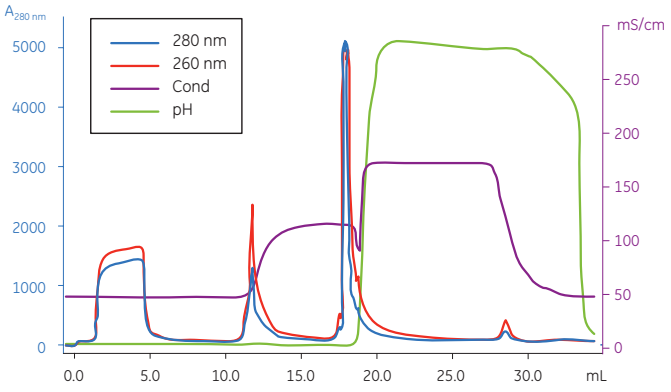

Fig 4. Chromatogram from the life-cycle study showing UV curves from 10 runs with sample using Capto ViralQ, UV curves at 280 nm (blue) and 260 nm (red), conductivity (lilac), and pH (green).

There was no significant difference between the 10 runs. The recovery of HA was on average 88% with a 3-fold log reduction of gDNA. The amount of gDNA recovered in the elution peak was consistent in all runs.

Linear fluid velocities

We used a standard flow rate of 60 cm/h (i.e., 20 min residence time). A higher flow rate of 300 cm/h was also tested on two occasions. The same column and starting material were used in all experiments.

Figure 5 shows that the different flow rates produced similar results and both provided a high recovery of virus (HA) in the flowthrough. A slight increase in the level of gDNA was observed in the flowthrough with the higher flow rate.

Column: Capto ViralQ (CV = 2 × 20 mL, XK16 column)
Buffer A: 20 mM Tris, 0.5 M NaCl, pH 7.5
Buffer B: 20 mM Tris, 1.5 M NaCl, pH 7.5
Sample: 2 CV concentrated Influenza A/PR/8/34 (H1N1), produced in MDCK cells
Flow: 60 cm/h and 300 cm/h

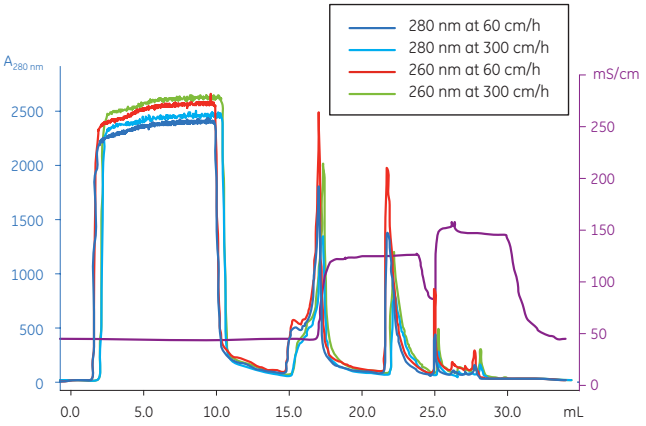

Fig 5. Chromatogram showing UV curves at 280 nm (blue) and 260 nm (red) for 60cm/h linear flow velocity and at 280 nm (light blue) and 260 nm (green) for 300 cm/h linear flow velocity.

Table 1. The table shows analytical results of HA (SRID) recovery and DNA reduction from three runs using a Capto ViralQ 1 mL HiTrap column. Start = Start sample for the run, FT = Flowthrough peak and Elu = Elution peak

Step		Volume (mL)	HA (SRID) conc. (µg/mL)	HA (SRID)recovery (%)	qPCR DNA conc. (ng/mL)	qPCR DNA recovery (%)	DNA log reduction
Start		2.5	67	100	61917	100	
Run 1:	FT	4	33	79	36	0	3.2
Run 5:	FT	4	40	96	44	0	3.1
Run 10:	FT	4	38	91	40	0	3.2

Table 2. The table shows analytical results of HA and DNA from runs using a Capto ViralQ XK16 column (CV = 20 mL,) with two different batches of A/Solomon Islands/3/2006 (H1N1) influenza virus. FT = Flowthrough peak

Harvest	Linear fluid velocity (cm/h)	HA conc. SRID (µg/mL)		HA yield in FT (%)	DNA conc. QPCR (ng/mL)		DNA Log reduction in FT
		Start	FT	SRID	Start	FT	FT
Batch 1	60	85*	61	86	7596	60	2.0
Batch 1	300	152*	111	88	8805	120	1.8
Batch 2	60	60	48	96	2012	< 35	1.8
Batch 2	300	60	52	104	2012	< 35	1.8

* SRID has a high variability—these samples were analyzed at two different occasions with several days in between.

Table 3. The table shows analytical results of of HA and DNA from runs using a Capto ViralQ XK16 column (CV = 20 mL,) with A/Solomon Islands/3/2006, A/Wisconsin/67/2005, and B/Malaysia/2506/2004 influenza virus. Start = Start sample for the run, FT = Flowthrough peak and Elu = Elution peak

Influenza strain	HA conc. SRID (µg/mL)			HA yield in FT (%)	DNA conc. qPCR (ng/mL)			DNA Log reduction factor
	Start	FT	Elu	SRID	Start	FT	Elu	FT
Influenza H1N1	60	40	< 6	80	2012	< 35	2859	1.8
Influenza H3N2	28	23	< 6	99	11 253	< 35	11 716	2.4
Influenza B	15	7	< 6	82	96 813	15	90 102	3.8

Three seasonal influenza strains

The effectiveness of gDNA clearance was confirmed by repeating the experiment with the A/Solomon Islands/3/2006 (H1N1) strain and two additional strains of the seasonal influenza virus A/Wisconsin/67/2005 (H3N2) and B/Malaysia/2506/2004.

All three strains of the influenza virus were produced in MDCK cells, clarified, and concentrated before running on a Capto ViralQ column. Table 3 shows the yield of HA and the amount of gDNA in the flowthrough for the three strains.

Conclusions

The gDNA scavenging method described in this application note provides consistent results with regard to the reduction of genomic DNA from cell-cultured influenza virus at different conditions and with the three strains that we tested.

In addition, the method enables efficient purification with a recovery of > 80% (HA), as determined by SRID.

The gDNA scavenging method described in this application note is a simple and scalable protocol for the production of influenza virus.

Ordering information

Product	Quantity	Code number
Capto Q	100 mL	17-5316-02
Capto Q	1 L	17-5316-03
Capto Q	5 L	17-5316-04
Capto Q	10 L	17-5316-05
HiTrap Capto Q	5 × 1 mL	11-0013-02
HiTrap Capto Q	5 × 5 mL	11-0013-03
HiScreen™ Capto Q	1 × 4.7 mL	28-9269-78

We kindly ask you to change to the corresponding product, Capto Q, that contains the same chromatography medium as Capto ViralQ.

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