# Removal of DNA and baculovirus from influenza virus-like particles using Capto Q

**Intellectual Property Notice:** The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.

**cytiva.com**



CY13365-12May20-AN

# Removal of DNA and baculovirus from influenza virus-like particles using Capto™ Q

**This application note presents a chromatographic step for efficient removal of DNA and baculovirus (BV) from influenza virus-like particle (VLP) supernatant solutions, manufactured using Novavax Inc. proprietary VLP technology. The chromatographic step uses Capto Q medium in flowthrough mode so that the VLPs pass through the column while the contaminants are retained; a method that allows higher stability of the product. Using Capto Q in this application enables higher productivity and time saving by allowing linear fluid velocities up to at least 300 cm/h during loading and 600 cm/h during desorption. The method was developed and tested for the purification of avian influenza H5N1 and swine influenza H1N1 VLPs at laboratory scale. The chromatographic step was successfully scaled up for H1N1 influenza VLPs to pilot scale in an AxiChrom™ 70 column. This represents a 160-fold volumetric scale-up from the development column scale.**

## Introduction

Influenza infection remains a major threat to human health. One solution is the use of VLPs that contain one or more viral structural proteins organized in a similar manner as in native viruses with surface proteins displayed in an immunologically correct conformation (Fig 1). VLPs can elicit protective immune responses and thus have the potential to be a solution to the vaccine production problem. The work presented in this application note was performed jointly with Novavax Inc., a clinical stage biopharmaceutical company creating novel vaccines to address a broad range of infectious diseases worldwide using advanced proprietary VLP technology.

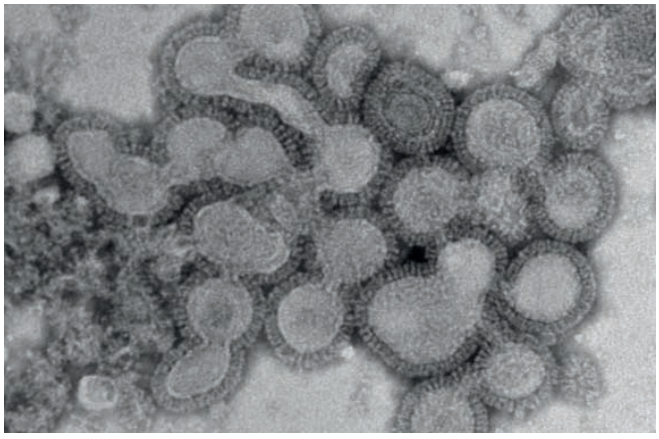


**Fig 1.** Electron micrograph of VLPs.

The production of influenza VLPs bearing the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) is achieved using a baculovirus (BV) insect cell expression system with cells derived from, for example, *Spodoptera frugiperda* (Sf-9). The insect cells are infected with a recombinant BV that contains artificially introduced foreign nucleic acid sequences encoding for the desired influenza proteins. After incubation, influenza VLPs are released into the cell supernatant, inevitably accompanied by impurities, mainly host cell DNA and BV, that need to be efficiently removed in order to obtain VLPs of clinical grade.

This Application note presents a chromatographic step to efficiently remove DNA and BV from influenza VLP supernatant solutions. Capto Q medium is used in flowthrough mode, allowing the VLPs to pass through the column while contaminants are retained; a method that will allow higher retention of particles. This method has been developed for the purification of avian influenza H5N1 and swine influenza H1N1 VLPs at laboratory scale and has been successfully scaled up to an AxiChrom 70 column (≈ 800 mL of medium).

## Materials and methods

ÄKTAexplorer™, ÄKTApilot™, HiTrap™ Capto Q columns, HiPrep™ Capto Q columns, AxiChrom columns, and Capto Q medium were from GE Healthcare. Tris buffer was used with low or high salt.

For the chromatography experiments, ÄKTAexplorer or ÄKTApilot (used with AxiChrom column) were used with UNICORN™ v5.11 software. Purifications were monitored by UV. After each chromatography run, the columns were subject to CIP using the following procedure: 1 column volume (CV) of 1 M NaOH at 30 cm/h, 1 h pause, 1 CV of 1 M NaOH at 30 cm/h, 3 CV of 2 M NaCl at 30 cm/h, 3 CV of purified water at 90 cm/h, and finally 2 CV of 20% ethanol at 90 cm/h.

### *Cell culture and infection*

Both influenza VLPs (H5N1 and H1N1) were produced by infection of Sf-9 cells with recombinant BVs. After incubation and harvest, VLP feed was prepared in low-salt buffer. Prior to use, VLP concentration was estimated as absorbance at 280 nm.

### *Method development using HiTrap Capto Q*

The loading capacity was tested by loading a fixed volume of VLP feed onto prepacked 5 mL HiTrap Capto Q columns that had been preequilibrated with low salt buffer. For H5N1 VLP, two experiments were performed where 5.2 and 7.8 absorbance units (AU)/mL medium, respectively, were loaded onto the HiTrap Capto Q column. The flow rate was 3 mL/min (90 cm/h). After loading, the column was washed with 2 CV of low-salt buffer, after which elution of BV and DNA was performed by increasing the salt concentration in the buffer up to 1.5 M NaCl with a 5 CV linear gradient from 0% to 100% high-salt buffer, after which the column was washed with purified water (3 CV). For H1N1 VLP, an analogous series of experiments was performed where VLPs corresponding to 2.0, 4.0, and 5.3 AU/mL medium were loaded as described for H5N1. The flow rate was lower, 1.5 mL/min (45 cm/h).

Recovery tests were run on 5 mL HiTrap Capto Q columns by loading VLP feeds at two different flow rates. The VLP feeds (4 AU/mL medium) were loaded at 1.25 mL/min (37.5 cm/h) or 0.19 mL/min (5.7 cm/h), corresponding to a residence of 4 and 26 min, respectively. Once the loading was complete, the column was washed with low-salt buffer, and elution of BV and DNA was achieved by a linear gradient increasing the NaCl concentration in the mobile phase up to 1.5 M, after which the column was washed with 3 CV of purified water.

### *Optimization in HiPrep 16/10 Capto Q**

Optimization work was done using two HiPrep 16/10 Capto Q columns connected in series (20 cm bed height for a total volume of 40 mL) loaded with VLP feed (4 AU/mL medium) at 4 and 26 min residence time. Once loading was complete, the columns were washed with low-salt buffer, and elution of BV and DNA was achieved by a linear gradient increasing the NaCl concentration in the mobile phase up to 1.5 M. After elution, the column was washed with 3 CV of purified water.

*Produced on customer request

### *Linear scale-up to AxiChrom 70/300*

Four consecutive scale-up runs of H1N1 VLP feed were performed on an AxiChrom 70 column packed to a bed height of 21 cm (808 mL volume) operated by ÄKTApilot system. The whole procedure was run at 300 cm/h (approx. 4 min residence time) and fractions were collected manually; 400 mL of sample (3.64 AU/mL medium) were loaded in each run. Samples were taken for analysis from the VLP pools and the BV and DNA peaks.

### *SDS-PAGE*

Reduced SDS-PAGE was run according to Novavax standard operating procedure using a 4% to 12% polyacrylamide gel (NuPaGE™ precast mini gels, Invitrogen). Proteins were fixed and visualized by Coomassie™ staining (GelCode™ Blue Strain Reagent, Thermo Scientific).

### *Enzyme-linked immunosorbent assay (ELISA)*

The quantitation of BV-specific antigen in the VLP fractions was done by an ELISA according to a Novavax standard operating procedure.

### *Single radial immunodiffusion (SRID)*

SRID was used to quantitate the amount of antigenic HA present in the samples. This analysis was run according to GE Healthcare standard operating procedures, using GE Healthcare ultrapure agarose and a CyDye™ fluorescent labeling kit to reveal the gel. Diffusion was allowed to proceed overnight at room temperature in a dark room.

### *DNA assays*

Detection of DNA in VLP fractions was achieved using agarose gel electrophoresis (AGE), followed by visualization of DNA using ethidium stain and a Typhoon™ 9410 scanner. In some experiments, quantitation of DNA in samples was performed using the PicoGreen DNA assay (Invitrogen).

# Results and discussion

## *Capacity testing*

Sample load optimization was performed using HiTrap Capto Q (Fig 2). To detect any BV impurities, the flowthrough (VLP) fractions from each experiment were collected and analyzed by SDS-PAGE (Fig 3). The lower load of 5.2 AU/mL medium seemed suitable for effective BV removal. In the H1N1 VLP fractions (Fig 2B), no BV could be detected for any of the loaded concentrations. For H5N1 (Fig 2A), a BV band appears in the tailing part of the VLP fraction from the higher load experiment (7.8 AU/mL medium), indicating that the column has been overloaded.

A complementary BV ELISA analysis for quantitative assessment of the BV removal was carried out for each VLP pool. When loading 4.0 AU/mL medium (280 nm); at this load, 93% of BV in the sample was removed (data not shown). This load therefore appears to be optimal for H1N1 VLPs. AGE analysis was performed to detect DNA in the flowthrough peaks. Within the sensitivity limits of this assay, no DNA was found in any fraction, indicating that column overload mainly resulted in increased levels of BV (data not shown).

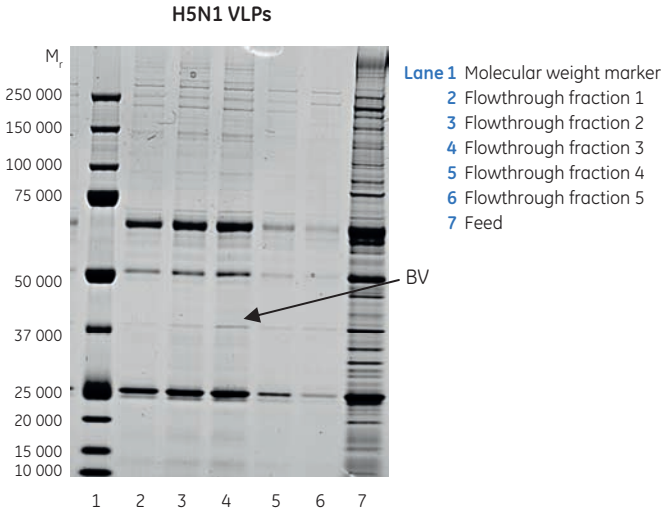



**Fig 3.** SDS-PAGE fraction profiles from H5N1 chromatogram in Figure 2A. Breakthrough of BV is indicated with an arrow.

## *Recovery testing*

After the optimum quantity of feed to be loaded had been determined, the recovery of the two VLPs was studied at two different residence times each. Chromatograms from the recovery test of H5N1 are shown in Figure 4.

A)

| **Running conditions** | |
|---|---|
| *Column:* | 5 mL HiTrap Capto Q |
| *Sample:* | H5N1 VLP feed |
| *Sample load:* | 5.2 and 7.8 AU/mL medium |
| *Buffer A:* | Low-salt Tris buffer |
| *Buffer B:* | High-salt Tris buffer |
| *Wash after sample:* | 2 CV buffer A |
| *Flow rate:* | 3 mL/min |
| *Linear gradient:* | 0% to 100% buffer B in 5 CV |
| *System:* | ÄKTAexplorer 100 |

B)

| **Running conditions** | |
|---|---|
| *Column:* | 5 mL HiTrap Capto Q |
| *Sample:* | H1N1 VLP feed |
| *Sample load:* | 2.0, 4.0, and 5.0 AU/mL medium |
| *Buffer A:* | Low-salt Tris buffer |
| *Buffer B:* | High-salt Tris buffer |
| *Wash after sample:* | 2 CV buffer A |
| *Flow rate:* | 3 mL/min |
| *Linear gradient:* | 0% to 100% buffer B in 5 CV |
| *System:* | ÄKTAexplorer 100 |

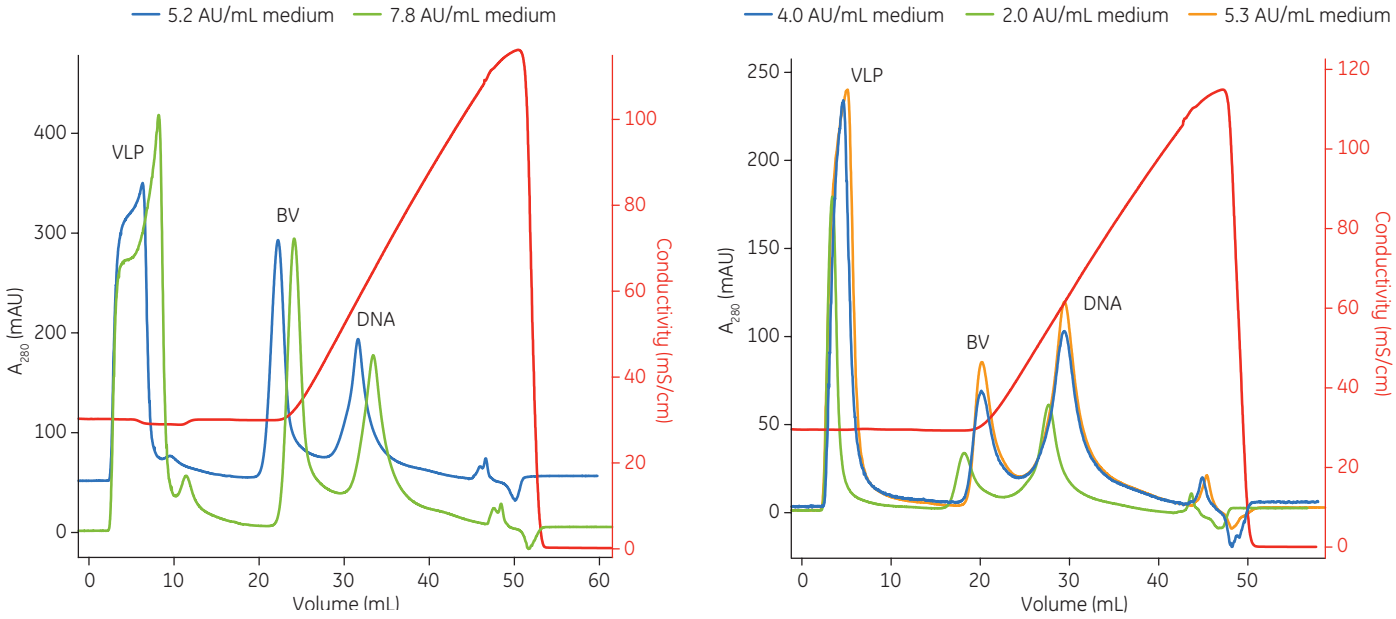



**Fig 2.** Capacity testing of **(A)** H5N1 VLP at 5.2 and 7.8 AU/mL medium and **(B)** H1N1 VLP at 2.0, 4.0, and 5.3 AU/mL medium. 50 mAU offset between curves.

**Running conditions**

| | | | |
|---|---|---|---|
| *Column:* | 5 mL HiTrap Capto Q | *Wash after sample:* | 2 CV buffer A |
| *Sample:* | H5N1 VLP feed | *Flow rate:* | 0.19 or 1.25 mL/min |
| *Sample load:* | 4 AU/mL medium | *Linear gradient:* | 0% to 100% buffer B in 5 CV |
| *Buffer A:* | Low-salt Tris buffer | | |
| *Buffer B:* | High-salt Tris buffer | *System:* | ÄKTAexplorer 100 |

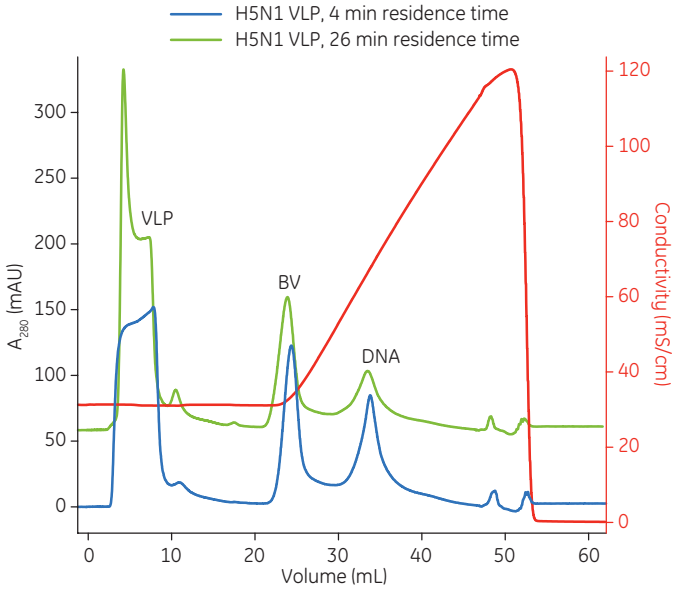



**Fig 4.** Recovery testing of VLPs run on 5 mL HiTrap Capto Q column for 4 and 26 min residence time, respectively; 50 mAU offset between curves.

The purity of the collected VLP fractions was analyzed by SDS-PAGE (Fig 5). The gels show almost identical band profiles between the two residence times for both H5N1 VLPs and H1N1 VLPs.

All VLPs in the flowthrough fraction were collected and the recovery was calculated after analysis with SRID (Table 1). For H5N1, an increased residence time lowered the recovery; a residence time of 26 min resulted in a recovery of 65.4%, which is below the set specification of 70%, while a shorter residence time of 4 min resulted in a recovery of 80.4%. The recovery of H1N1 VLP on the other hand was not affected by residence time. For H1N1 VLPs, DNA concentration was also measured using PicoGreen kit and no significant amounts of DNA could be detected. The recovery testing showed that the yield of H5N1 VLPs increased with higher flow rate while no effect was seen for H1N1 VLPs. Thus, the set residence time for the step was 4 min.

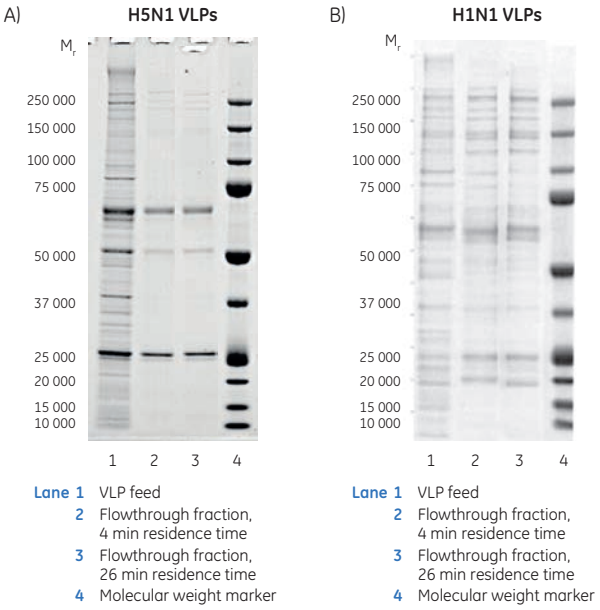



**Fig 5.** SDS-PAGE after Capto Q purification of **(A)** H5N1VLP **(B)** H1N1 VLP.

## Optimization in HiPrep 16/10 Capto Q column

In order to confirm the chromatographic conditions established on 5 mL HiTrap columns, the separation conditions and methods were tested on HiPrep 16/10 Capto Q. Chromatograms from scale-up runs on 2× HiPrep 16/10 Capto Q columns are shown in Figure 6. The purity of the collected VLP peaks was checked by SDS-PAGE analysis (Fig 7). For both VLP constructs, no significant difference between the two residence times could be observed. H5N1 gives an initial spike in the chromatogram (Fig 6A); it was determined that this spike had the same composition as the rest of the peak (Fig 7A).

The recoveries in the collected flowthrough fractions were calculated based on SRID analysis (Table 2). As could be seen for HiTrap columns, a shorter residence time gave better recovery for H5N1 VLPs, while residence time has no influence on recovery or H1N1. DNA concentrations in the H1N1 flowthrough fractions were measured using Picogreen. The purity and recovery determined in the HiPrep 16/10 Capto Q column scale-up experiments are in accordance with those observed with HiTrap columns in a format with 20 cm bed height, which is the bed height desired in final use.

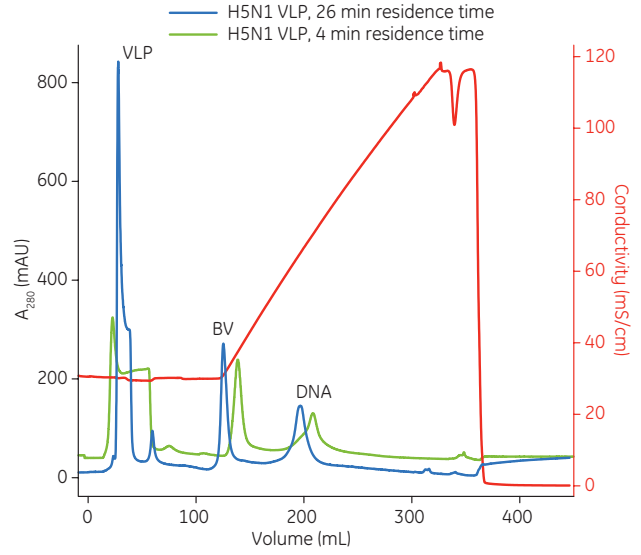
**Table 1.** Recovery dependence of sample residence time

| Sample | Residence time (min) | [HA] (µg/mL) | Vol (mL) | Recovery (µg HA) | Amounts applied (AU) | Recovery (%) |
|---|---|---|---|---|---|---|
| H5N1 VLP feed | | 81.7 | 5.6 | | | |
| H5N1 eluate from 5 mL HiTrap Capto Q (1.25 mL/min) | 4 | 53.3 | 6.9 | 370 | 4 | 80 |
| H5N1 eluate from 5 mL HiTrap Capto Q (0.19 mL/min) | 26 | 51.6 | 5,8 | 299 | 4 | 65 |
| H1N1 VLP feed | | 65.5 | 2.4 | | | |
| H1N1 eluate from 5 mL HiTrap Capto Q (1.25 mL/min) | 4 | 17.7 | 6 | 106 | 4 | 69 |
| H1N1 eluate from 5 mL HiTrap Capto Q (0.19 mL/min) | 26 | 19.3 | 6 | 116 | 4 | 75 |

A)

**Running conditions**

| | |
|---|---|
| *Column:* | 2 × HiPrep 16/10 Capto Q |
| *Sample:* | H5N1 VLP feed **(A)**, H1N1 VLP feed **(B)** |
| *Sample load:* | 4 AU/mL medium |
| *Buffer A:* | Low-salt Tris buffer |
| *Buffer B:* | High-salt Tris buffer |
| *Wash after sample:* | 2 CV buffer A |
| *Flow rate:* | 1.5 or 10 mL/min |
| *Linear gradient:* | 0% to 100% buffer B in 5 CV |
| *System:* | ÄKTAexplorer 100 |




B)

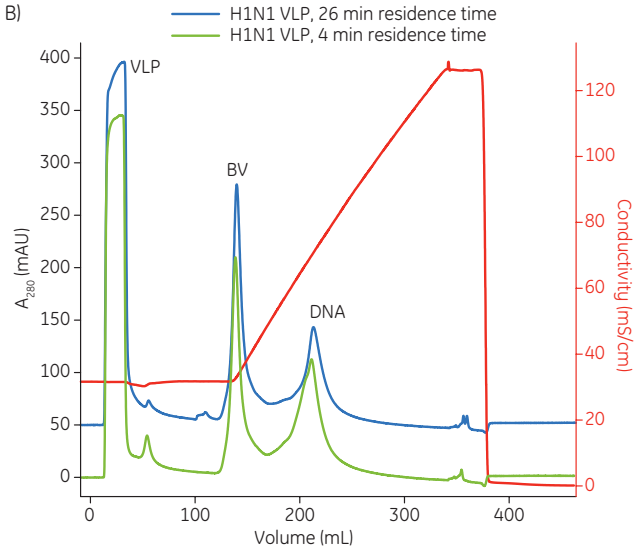
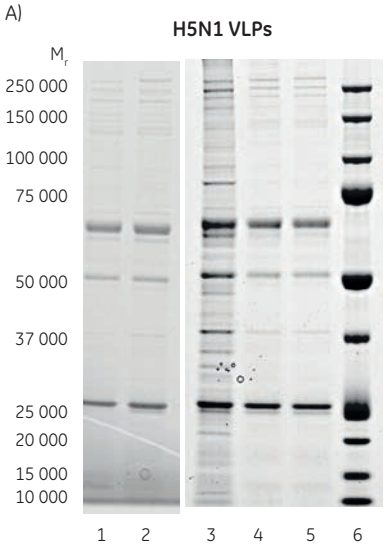


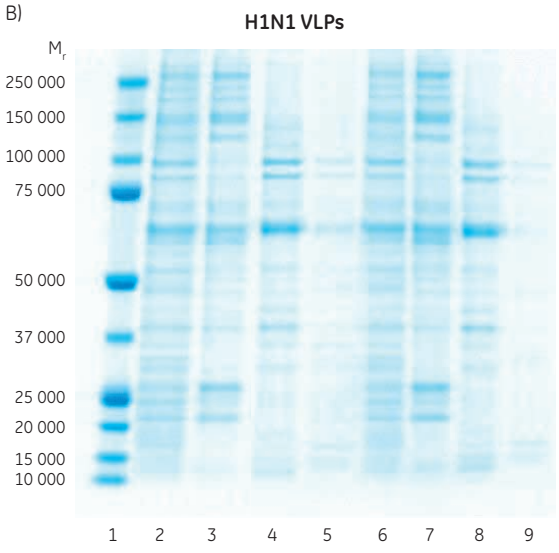

**Fig 6.** Scale-up of purification of VLP feed on HiPrep 16/10 Capto Q columns **(A)** H5N1. The spike at the beginning of the VLP peak consists exclusively of VLP material as demonstrated in Figure 7. **(B)** H1N1. Absorbance measured at 280 nm; 50 mAU offset between curves.

A)




**Lane 1** Flowthrough fraction, 26 min residence time, spike
**2** Flowthrough fraction, 26 min residence time
**3** VLP feed
**4** Flowthrough fraction, 4 min residence time, spike
**5** Flowthrough fraction, 4 min residence time
**6** Molecular weight marker

B)




**Lane 1** Molecular weight marker
**2** VLP feed
**3** Flowthrough fraction, 4 min residence time
**4** BV peak, 4 min residence time
**5** DNA peak, 4 min residence time
**6** VLP feed
**7** Flowthrough fraction, 26 min residence time
**8** BV peak, 26 min residence time
**9** DNA peak, 26 min residence time

**Fig 7.** SDS-PAGE analysis of scale-up runs with **(A)** H5N1 VLP feed and **(B)** H1N1 VLP feed on HiPrep 16/10 Capto Q column.

**Table 2.** VLP recovery results from scale-up runs on HiPrep 16/10 Capto Q columns

| Sample | Residence time (min) | [HA] (µg/mL) | Vol (mL) | Recovery (µg HA) | Amounts applied (AU) | Recovery (%) |
|---|---|---|---|---|---|---|
| H5N1 eluate from 2 × HiPrep 16/10 Capto Q (1.5 mL/min) | 4 | 63.6 | 41.5 | 2640 | 4 | 74 |
| H5N1 eluate from 2 × HiPrep 16/10 Capto Q (10 mL/min) | 26 | 65.6 | 25.4 | 1768 | 4 | 51 |
| H1N1 eluate from 2 × HiPrep 16/10 Capto Q (1.5 mL/min) | 4 | 18.4 | 30 | 552 | 4 | 65 |
| H1N1 eluate from 2 × HiPrep 16/10 Capto Q (10 mL/min) | 26 | 20.6 | 30 | 618 | 4 | 60 |

## Scale-up on AxiChrom 70 column

To verify the scalability of the step before technical transfer to production, a limited scale-up to pilot scale in AxiChrom 70 was done. This represents a 160-fold volumetric scale-up from the development column and a 20-fold linear scale-up from the verification scale in HiPrep 16/10 Capto Q. Four consecutive runs were performed with H1N1 VLP feed (Fig 8) and the recovery figures are presented in Table 3. The results show that the scale-up was successful, with retained resolution and reduction of impurities. Yields were unexpectedly low in the scale-up runs. This was traced to the composition of the specific upstream batch of starting material used for the scale-up.

| | |
|---|---|
| *Column:* | AxiChrom 70/300, packed with 808 mL Capto Q |
| *Sample:* | H1N1 VLP feed |
| *Sample load:* | 400 mL sample (3.64 AU/mL medium) |
| *Buffer A:* | Low-salt Tris buffer |
| *Buffer B:* | High-salt Tris buffer |
| *Wash after sample:* | 2 CV buffer A |
| *Flow rate:* | 1.5 or 10 mL/min |
| *Linear gradient:* | 0% to 100% buffer B in 5 CV |
| *System:* | ÄKTApilot |

**UNICORN method**

| | |
|---|---|
| *Preequilibration:* | 1 CV buffer B |
| *Equilibration:* | 2 CV buffer A |
| *Sample application:* | 0.5 CV |
| *Wash:* | 2 CV buffer A |
| *Gradient:* | 5 CV 0% to 100% buffer B |
| *Hold:* | 1 CV buffer B |
| *Purified water:* | 3 CV |

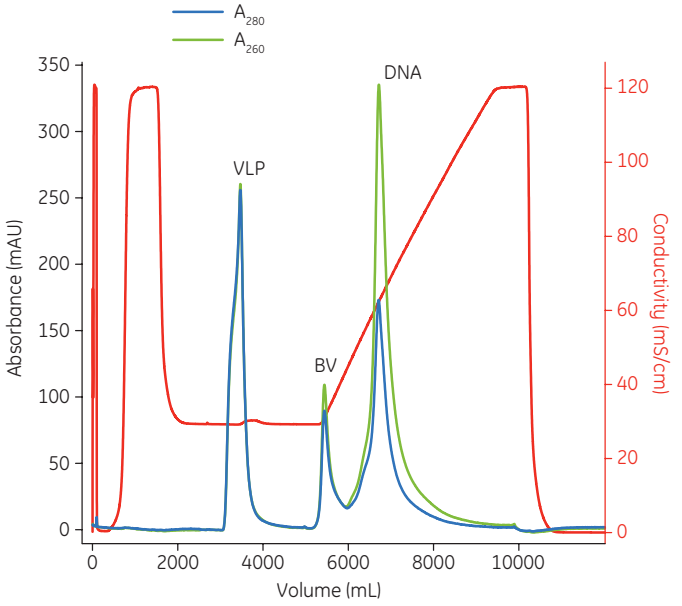



**Fig 8.** Representative scale-up chromatogram for H1N1 VLP feed.

## CIP procedure

An efficient and economical cleaning of the column is essential to maintain an economical lifecycle for the medium, as well as good process economy. A typical chromatogram of a CIP procedure is shown in Figure 9. Almost all impurities are removed after 1 M NaOH treatment since no more absorption is observed following the other washes. As indicated by the higher absorption at 260 nm, DNA appeared to be the main contaminant removed during this procedure. When this CIP procedure was used after each run, at least 12 separations could be carried out on the same column without observing any performance loss. The CIP procedure developed fulfills the set specifications while being fast and using a simple combination of chemicals.

| | |
|---|---|
| *Column:* | AxiChrom 70/300, packed with 808 mL Capto Q |
| *Eluents:* | 1 M NaOH (1CV)<br>2 M NaCl (3CV)<br>Purified water (3 CV)<br>20% ethanol (2 CV) |
| *Flow rate:* | 30 cm/h or 90 cm/h (water)<br>1 h pause after 1 M NaOH |
| *System:* | ÄKTApilot |

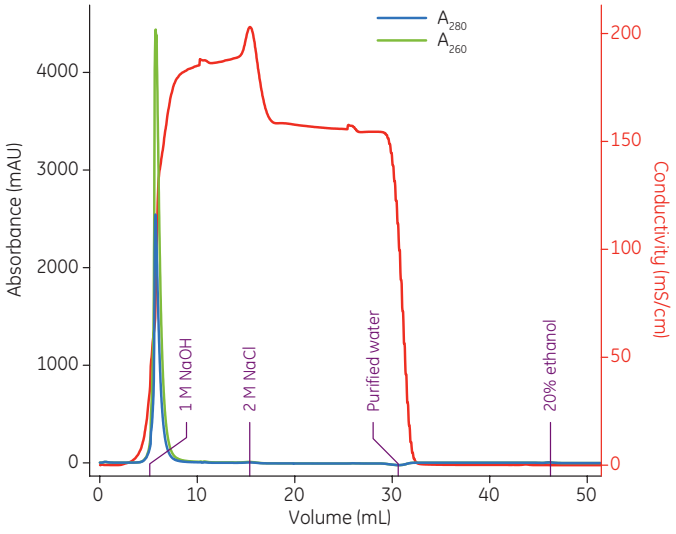



**Fig 9.** Typical chromatogram of a CIP procedure.

**Table 3.** Recovery results from scale-up run on AxiChrom 70 Capto Q

| Sample | [HA] (µg/mL) | Vol (mL) | Recovery (µg HA) | Amounts applied (AU) | Recovery (%) |
|---|---|---|---|---|---|
| H1N1 VLP | 92.2 | 400 | | | |
| AxiChrom Capto Q run 1 (VLP peak) | 25.3 | 601 | 15805 | 3.64 | 43 |
| AxiChrom Capto Q run 2 (VLP peak) | 28.1 | 577 | 16213 | 3.64 | 44 |
| AxiChrom Capto Q run 3 (VLP peak) | 25.8 | 592 | 15895 | 3.64 | 43 |
| AxiChrom Capto Q run 4 (VLP peak) | 24.6 | 588 | 14465 | 3.64 | 39 |

## Conclusion

An initial chromatography step using Capto Q in flowthrough mode allows removal of significant amounts of BV and DNA from influenza VLPs. The efficiency of BV removal depends on the VLP feed and the relative amounts of impurities found in this feed. The described procedure offers an excellent method to be integrated in a vaccine downstream process and can be combined with a polishing step to remove any remaining impurities. The use of flowthrough mode allows for higher yield of intact VLPs compared to binding chromatography, since binding involves shear forces and conformational changes that can affect VLP stability. Using flowthrough mode also allows the procedure to be used for different VLPs with minimal changes to the method, allowing a platform approach.

The use of Capto Q allows high linear velocities, up to at least 300 cm/h during loading and 600 cm/h during desorption, which improves process productivity. The efficient CIP methodology permits multiple reuse of the medium without any dramatic decrease of its purification ability. The described method is scalable across different column formats, from HiTrap to AxiChrom 70. As the AxiChrom platform offers scalable and predictable performance this method can easily be further scaled up to manufacturing scale.

## Ordering information

| Product | Quantity | Code no. |
|---|---|---|
| HiTrap Capto Q | 5 × 5 mL | 11-0013-03 |
| HiPrep Capto Q* | – | 28-9375-48 |
| Capto Q | 1 L | 17-5316-03 |
| AxiChrom 70/300 | – | 28-9018-40 |

*Produced on customer request

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

For local office contact information, visit
**www.gelifesciences.com/contact**

www.gelifesciences.com/bioprocess

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

imagination at work

28-9924-41 AA 05/2011