# Concentration and diafiltration of cell-derived, live influenza virus using 750 C hollow fiber filter cartridge

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# Concentration and diafiltration of cell-derived, live influenza virus using 750 C hollow fiber filter cartridge

**This application note describes the performance of GE Healthcare Life Sciences 750 C hollow fiber filter cartridge in concentration and diafiltration (buffer exchange) of cell culture-derived, live influenza virus. The 750 C cross flow filter is designed for cross flow filtration (concentration, impurity removal, and buffer exchange) of influenza virus and other entities of similar size. In this study, the influenza harvest was clarified using a normal flow filtration step, and then concentrated and diafiltered using the 750 C filter cartridge. Comparative experiments were performed with 500 C hollow fiber filter cartridge to evaluate differences in impurity removal capacity and flow characteristics. Intermediate and final fractions were analyzed for purity and yield of the target product. The well-defined molecular weight cut-off of the filter membrane allows separation of virus from impurities. Feed concentration and buffer exchange can be performed sequentially using the same equipment. The results show that both 500 C and 750 C hollow fiber filters enable achieving high virus yields (> 80%) and similar host cell protein (HCP) removal. However, the more open structure of the 750 C filter membrane resulted in a 1.5 to 2 order of magnitude higher removal of host cell DNA (hcDNA) compared with the 500 C filter.**

## Introduction

Cross flow filtration (CFF) is a technique extensively used in vaccine production and particularly in influenza vaccine manufacturing. In contrast to normal flow (dead-end) filtration (NFF), the feed is recirculated over a permeable membrane surface (cross flow). In CFF, liquid and compounds with molecular weights ($M_r$) less than the membrane cut-off can pass through the membrane (permeate), whereas larger molecules are retained and concentrated (retentate).

**Fig 1.** Hollow fiber filter cartridges.

For a shear-sensitive target such as influenza virus, a hollow fiber cartridge is commonly used for the CFF step. A hollow fiber filter usually causes less damage to the target product compared with a flat sheet membrane. In addition, the high membrane strength and temperature stability of hollow fiber filters allow for sterilization by autoclaving.

Hollow fiber filters consist of a bundle of parallel, permeable polysulfone fibers. The filters are provided with a broad range of lumen diameters, pore sizes, and surface areas (Fig 1). The filter size range enables scalable processes, from small laboratory scale to large manufacturing scale.

Larger lumen sizes are typically used when processing viscous or particle-laden fluids such as whole cells or lysates. A larger lumen diameter minimizes pressure build-up with increasing concentration factor. Small lumen sizes are better suited for low-viscosity, clean fluids such as concentration and diafiltration of proteins.

**Table 1.** Comparison between hollow fiber filters of different i.d.

| Fiber i.d. | Membrane area | No of devices | Shear rate | Recirculation flow rate |
|---|---|---|---|---|
| 0.5 mm | 24.4 $m^2$ | 4 size 65 cartridges | 16 000 $s^{-1}$ | 316 L/min |
| 1.0 mm | 26.4 $m^2$ | 6 size 65 cartridges | 16 000 $s^{-1}$ | 1470 L/min |

**Note**: shear rate is directly proportional to the recirculation flow rate. Thus, for a shear rate of 8000 $s^{-1}$, the recirculation flow rates would be half of the values listed above.

Recirculation flow rate is a strong contributor to system capital costs and varies significantly depending on the inner diameter of the hollow fiber membrane. As an example, for two processes with similar membrane surface areas and path lengths, and maintaining an equal shear rate, systems incorporating 0.5 mm i.d. (C-lumen) fibers require recirculation flow rates of less than one-fourth that of systems using 1 mm i.d. (E-lumen) fibers (Table 1). Thus, benefits of using filters with the smaller fiber i.d. 0.5 mm include reduced system cost, smaller system footprint, and a reduced system minimum void volume that can help achieving required concentration factors.

The 750 C hollow fiber filter cartridge, with a 750 000 nominal molecular weight cutoff (NMWC), is designed for use in virus purification workflows, and effectively removes ovalbumin and other proteins in allantoic fluid from egg-based virus production as well as host cell-derived impurities from production in mammalian cells. The 750 C filter cartridge can also be used for filtration of other entities of similar size.

In this work, the performance of 750 C was compared with that of 500 C hollow fiber filter with the same 0.5 mm lumen diameter but with a 500 000 NMWC. The filters were used in virus concentration and diafiltration and compared with regards to impurity removal and pressure/flow characteristics. Influenza virus, produced in MDCK cells, was used as model system.

## Materials and methods

An overview of the virus production process is given in Figure 2.

### *Influenza virus production and clarification*

Madin-Darby canine kidney (MDCK) cells were inoculated at 450 000 cells/mL in a bioreactor (Applikon Technology). The cells were cultured on Cytodex™ 3 microcarriers (5 g/L) in DMEM/F-12 medium supplemented with 5% fetal bovine serum for 48 h. At a density of approximately 1.9 × $10^6$ cells/mL, the culture medium was changed to serum-free DMEM/F-12 and the cells were infected with influenza A/Solomon Island/3/2006 (H1N1) virus.

Virus was harvested 72 h post infection. The harvest was clarified using serially connected 4" ULTA™ Prime GF 2 µm and 0.6 µm NFF filters to remove microcarriers, cells, and cell debris. The flux was 120 L/h/$m^2$. The clarified harvest was stored at 4°C until further use.

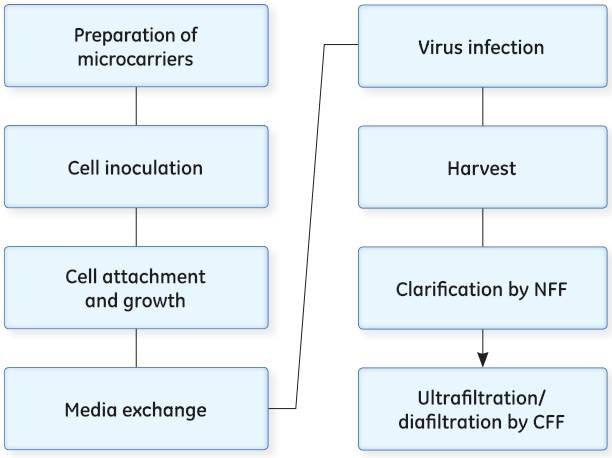


**Fig 2.** Schematic overview of the virus production process.

### *Concentration and buffer exchange*

#### Cross flow filtration

Filters used were Xampler™ hollow fiber cartridges size 3X2MA (290 $cm^2$, 63.5 cm, 30 lumen × 0.5 mm) with 500 000 and 750 000 NMWC. A filtration setup with permeate flux control was applied for the cross flow filtration step (Fig 3). The retentate was circulated at a shear rate of 5000 $s^{-1}$ (flow rate 157 mL/min). The transmembrane pressure (TMP) was maintained at 0.05 bar using a pinch valve on the retentate side. The permeate flux was set to 24 L/h/$m^2$ (flow rate 11.5 mL/min). The permeate flow was controlled by a two-channel peristaltic pump, using one channel for the permeate flow control and one channel for diafiltration buffer additions (20 mM phosphate, 150 mM NaCl, pH 7.2). A constant retentate volume (CRV) was maintained by continuous addition of diafiltration buffer. Feed, permeate, and flux were monitored through weight measurements. Running conditions are summarized in Table 2.

The retentate was over-concentrated with approximately 25 mL at the end, after which the system was emptied and the filter washed with 25 mL diafiltration buffer followed by 5 min circulation at 5000 $s^{-1}$ with the permeate flow closed. The wash was pooled with the retentate, resulting in a net concentration factor of 10 ×.

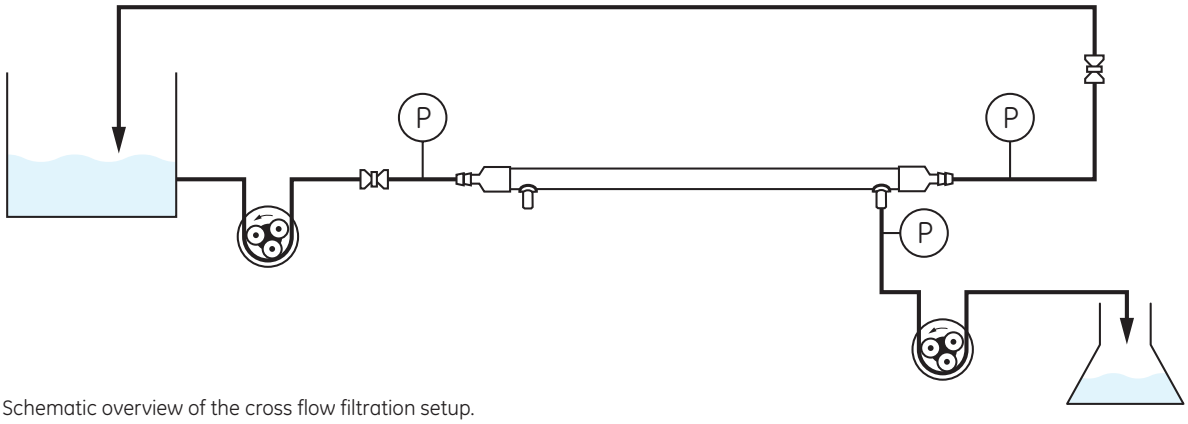


**Fig 3.** Schematic overview of the cross flow filtration setup.

**Table 2.** Running conditions for the concentration and diafiltration steps. Each filter type was run in duplicates.

| Hollow fiber cartridge | No of runs | Filter area ($cm^2$) | Start volumes (duplicate runs) (mL) | Concentration factor | Diafiltration factor | Filter load ($L/m^2$) | Retentate flow (mL/min) | Shear rate ($s^{-1}$) | TMP (bar) | Flux ($L/m^2/h$) |
|---|---|---|---|---|---|---|---|---|---|---|
| 500 C-3X2MA | 2 | 290 | 1299<br>1300 | 10 × | 10-fold | 84 | 157 | 5000 | 0.05 | 23 to 25 |
| 750 C-3X2MA | 2 | 290 | 1301<br>1301 | 10 × | 10-fold | 84 | 157 | 5000 | 0.05 | 23 to 25 |

### Cleaning-in-place (CIP) of filters after use

Hollow fiber cartridges can be cleaned and used several times. After use, filters were rinsed with 50°C $dH_2O$ and furthermore with 50°C 0.5 M NaOH, for approximately 60 min each. More details can be found in the operating handbook (18-1165-30). A water flux test was performed before use and after the CIP procedure.

## *Determination of virus content*

Samples taken after harvest, clarification, and during CFF were analyzed for virus activity by assaying 50% tissue culture infective dose ($TCID_{50}$) (1) and for hemagglutinin (HA) content using the Biacore™ T200 system (2). For the Biacore assay, recombinant HA protein was immobilized on the dextran matrix. Virus-containing samples were mixed with a fixed concentration of a virus-specific serum (NIBSC, Potters Bar, Hertfordshire, UK) and injected over the chip surface. Free antibodies (not bound to virus at equilibrium) bound to the surface HA, giving the response. More detailed descriptions are given in application notes 29-0435-48 and 28-9771-57.

## *Determination of impurities*

### hcDNA content

The hcDNA content was measured by quantitative PCR using primers specific for the MDCK cell genome. Sample preparation of hcDNA was performed using illustra™ GFX™ PCR DNA and Gel Band Purification Kit. MDCK cell genomic DNA was purified using illustra tissue and cell genomic Prep Mini Flow Kit and used as standards. The DNA concentration of the standard was determined with Quant-IT™ PicoGreen™ (Life Technologies Corp.)

### Total protein assay

Total protein content was measured with the Bradford total protein assay using BSA as standard (3).

### HCP content

HCP was quantitated with the Biacore T200 system using an in-house-produced polyclonal antibody raised against MDCK cell lysate. The antibodies were immobilized on the dextran matrix surface of the sensor chip. Samples were injected and HCP were allowed to bind to the immobilized surface antibodies, giving the response. A more detailed description is given in application note 28-9771-57.

# Results and discussion

## *Operating parameters*

Increasing the TMP increases the risk of membrane fouling, while higher shear rates tend to counteract fouling. According to previous experiments, a flux of 25 L/m²/h is appropriate for concentration and diafiltration of influenza feeds (see the operating handbook 18-1165-30 for more details). For processing of live influenza virus, it is important to handle the sample gently. To retain infectivity of the virus, the shear rate was set to 5000 $s^{-1}$. When processing inactivated influenza virus, on the other hand, it has been shown that the HA activity (red blood cell agglutination) is retained at shear rates up to 16 000$s^{-1}$ (4). However, for such high shear rates, the pressure gradient between the filter inlet and outlet is very high and strong pumps are required even for 0.5 mm i.d. C-lumen filters. As a controlled permeate flux usually generates higher productivity compared with an unrestricted flux, the permeate flux was controlled with a pump in this experimental setup.

As shown in Figure 4, the set parameters for shear rate (5000 $s^{-1}$) and flux (25 L/m²/h) resulted in steady processes with stable TMP during 10 × concentration and 10-fold diafiltration for both filters tested.

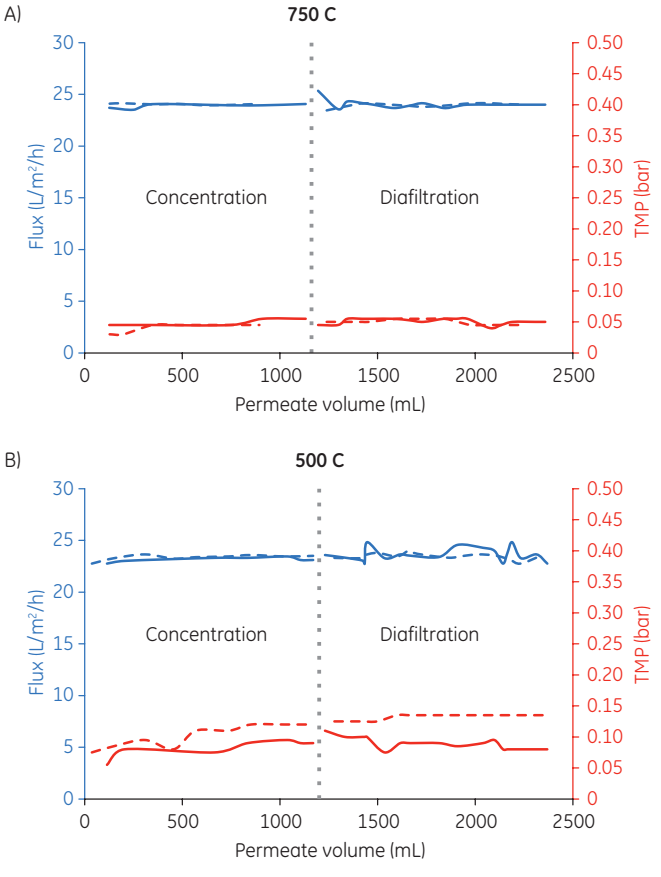


**Fig 4.** TMP (TMP = (($P_{in}$ + $P_{out}$)/2) – $P_{perm}$) and flux (L/m²/h) for concentration and diafiltration for (A) 750 C and (B) 500 C hollow fiber cartridges. Each figure displays duplicate runs, represented by solid and dotted lines.

## *Water flux tests*

The water flux test, performed in duplicate runs before use and after CIP, showed that approximately 80% of the water flux (L/m²/h per bar) was recovered after use for both 750 C and 500 C, respectively. The used CIP protocol operated very well for this feed stream of MDCK-produced influenza. More information on CIP protocols can be found in the operating handbook (18-1165-30).

## *Influenza virus yield and impurity removal*

Clarified harvest, containing 2.7 µg/mL HA, 99 µg/mL total protein, 149 µg/mL HCP, 4065 ng/mL hcDNA, and a $TCID_{50}$ of $10^{7.7}$ units/mL, was used as starting material.

Figure 5 illustrates the removal of impurities and retention of influenza virus over the whole CFF process for both filters. The two membranes 750 C and 500 C demonstrate comparable results regarding HA yields (80%) and protein removal.

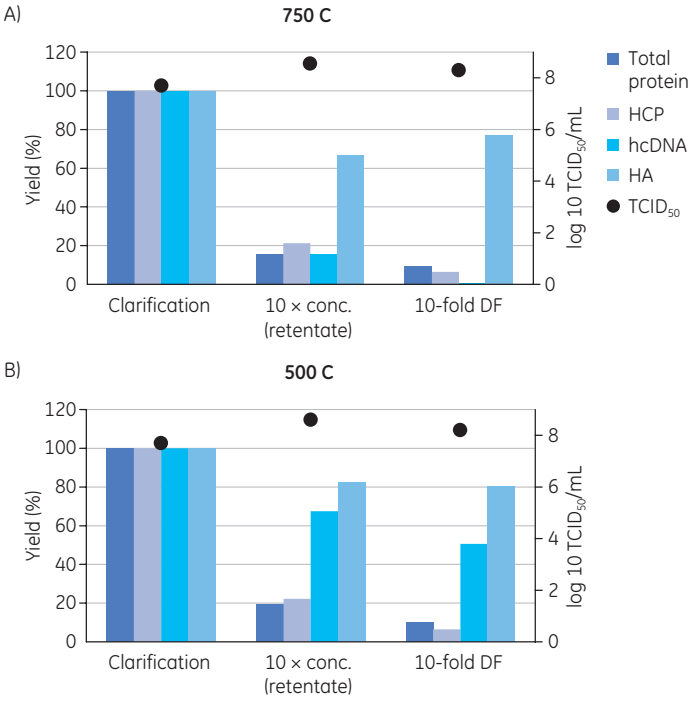


**Fig 5.** Virus yield (HA) and $TCID_{50}$ over the concentration and diafiltration (DF) process for (A) 750 C and (B) 500 C hollow fiber filters. Samples were analyzed after clarification, 10 × concentration, and 10-fold DF.

The $TCID_{50}$ assay is sensitive but has a high inherent variation, typically ± 1 order of magnitude. Hence, the yield (%) of infectious particles is not calculated. Virus content was instead determined as HA concentration. Although the high variation of the $TCID_{50}$ assay, the result indicates that one log higher $TCID_{50}$/mL was obtained after the 10 × concentration for both filters.

Approximately 80% HA was recovered after the initial concentration step. No infective virus, as determined by assaying $TCID_{50}$, was found in the permeate fractions for any of the two membranes. Stable $TCID_{50}$ and HA yields over the 10-fold diafiltration process were also shown for both membranes.

The Bradford total protein assay measures both HCP and virus protein, while the Biacore HCP assay is HCP specific. Approximately 80% of both HCP and total protein was removed in the concentration step and an additional 10% of each was removed during 4-fold diafiltration. An additional 1% to 2% of protein was removed after 10-fold diafiltration.

For the influenza feed used in this experiment, the main difference between the 750 C and 500 C filters was hcDNA removal. The 500 C filter showed 33% hcDNA removal in the concentration step, an additional 6% after 4-fold diafiltration, and an additional 4% after 10-fold diafiltration. For the 750 C filter, 85% of hcDNA was removed during concentration, an additional 13% after 4-fold diafiltration, and an additional 2% after 10-fold diafiltration.

The membrane structure and pore size of the 750 C filter are designed for virus particle retention and optimized for high HCP and hcDNA removal. The results show that the set parameters for shear rate (5000 $s^{-1}$) and flux (25 $L/m^2/h$) were appropriate for obtaining high yield of intact virus particles.

## Conclusion

In this application note, it is shown that influenza virus, produced in MDCK cell, can be processed to high purity and yield using the 750 C hollow fiber filter cartridge. The filter allowed retention of the virus particles, while removing impurities. More than 90% of the HCP was removed during the 10 × concentration and 10-fold diafiltration procedures. No loss of influenza virus infectivity was observed and approximately 80% of the HA was recovered in the retentate. A comparison between the 750 C and 500 C hollow fiber filters showed a 1.5 to 2 order of magnitude higher hcDNA removal using the more open 750 C membrane.

## References


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2. Estmer Nilsson, C., *et al.*, A novel assay for influenza virus quantification using surface plasmon resonance *Vaccine* **28**, 759–766 (2010).
3. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254 (1976).
4. Kalbfuss, B., *et al.*, Harvesting and concentration of human influenza A virus produced in serum-free mammalian cell culture for the production of vaccines. *Biotechnology and Bioengineering* **97**, 73–85 (2007).

# Order information

| Product | Model number | Pore size (NMWC) | Fiber i.d. (mm) | Membrane area ($cm^2$) | ($ft^2$) | Nominal flow path length (cm) | Code number |
|---|---|---|---|---|---|---|---|
| Xampler hollow fiber cartridge | UFP-500-C-3X2MA | 500 000 | 0.5 | 290 | 0.31 | 60 | 56-4101-53 |
| Xampler hollow fiber cartridge | UFP-750-C-3X2MA | 750 000 | 0.5 | 290 | 0.31 | 60 | 29-0110-51 |

| Related literature | Code number |
|---|---|
| Hollow fiber cartridges for membrane separations, operating handbook | 18-1165-30 |
| Downstream Scale-Up Purification of Influenza Virus Using ReadyToProcess™ Equipment, application note | 29-0435-48 |
| Biacore biosensor assays for quantitation of influenza virus and HCP, application note | 28-9771-57 |

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