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# Application Note

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## Minimizing Syringe Filter Usage in Harvesting Monoclonal Antibodies from CHO Cell Culture Supernatants

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### Abstract

Clarification of cell culture supernatants with volumes of < 25 mL for harvesting monoclonal antibodies by using syringe filters is often a laborious and, sometimes, an exhausting step. Therefore, selection of a suitable filter type is essential. In this study, we compared the performance characteristics of two suppliers' syringe filter types, each with a similar effective filtration area, for clarification of CHO cell culture supernatant samples. To obtain robust results, we examined ten combinations of cultivation methods and monoclonal antibody products, such as IgG1, IgG2, fc fusion proteins and bispecific antibodies, with regard to turbidity, mAb recovery, relative yield and throughput.
As a result, we found that syringe filter type Minisart® High Flow shows an average throughput of 18.0 mL compared with 9.3 mL of Acrodisc® at cell densities between $38.3 \times 10^5$ cells/mL and $163.6 \times 10^5$ cells/mL. For the other parameters, we did not find any significant differences. This finding emphasizes the importance of carefully selecting the syringe filter type to reduce the number of devices needed and thus workload.

## Introduction

Clarification of mammalian cell culture samples for preparative or analytical purposes is a necessary step to enable both a subsequent purification step and smooth operation of analytical instruments. The overall objective of the clarification step is to remove cells, cellular debris and other particles from the cell cultures while simultaneously allowing the target product to be recovered with a sufficient yield. The conventional procedure for clarification of small volumes (approx. < 25 mL) is a combination of centrifugation of the cell culture sample followed by microfiltration of the supernatant obtained. While centrifugation removes coarse and high-density particles, microfiltration is frequently necessary to eliminate small or low-density particles from the centrifugation supernatant. Microfiltration can serve simultaneously as a sterilization step by using sterilizing-grade filters rated to a pore size of 0.2 or 0.22 µm, called sterile filters.

Even though centrifugation removes the vast majority of particles, fine-pored filters tend to become clogged and often need to be changed out. As a result, this increases the number of filter devices used and compromises ergonomic handling. However, both the number of filters used and the associated operation time can be reduced by the correct choice of syringe filter.

In the present study, we show that the right choice of syringe filter for the clarification of CHO cell cultures can improve sample throughput and lower filter usage, without having a negative impact on turbidity, recovery of mAb product or total yield. Two common sterile syringe filters available on the market were selected with a pore size of 0.22 µm. Although slightly different in their effective filtration areas, they each had a polyethersulfone membrane.

## Methods and Materials

A comparative study was performed in an attempt to facilitate the filtration of cell culture supernatants for the development of cell lines. The syringe filter types Acrodisc® (Pall, order no. 4652, EFA = 5.8 $cm^2$) and Minisart® High Flow (Sartorius, order no. 16532-K, EFA = 6.2 $cm^2$) were examined regarding their filtration performance based on the following parameters: turbidity, mAb recovery, relative yield, throughput and filter usage. The study was conducted using CHO cell culture samples obtained from actual projects spread out over a period of three months.

In 13 cultivation batches, ten combinations of target proteins and cultivation methods were used (Table 1). In addition to 125 mL and 1,000 mL shaking flasks, 5 L stirred tank bioreactors (UniVessel, Sartorius) were also used. The cell density and viability were examined using the Vi-CELL XR supplied by Beckman Coulter. As target proteins, CHO cell lines were selected with mAbs from different IgG1 types, IgG2, fc fusion protein and from a bispecific antibody. The specific designation for the latter antibody is anonymized due to confidentiality agreements.

All cell culture batches were harvested after 14 days; two samples were taken from every batch (max. 31 mL per sample), one sample for clarification using Acrodisc® and the other using Minisart® High Flow. The samples were initially clarified by centrifugation for 60 min. at 4,000 g and the supernatants obtained were subsequently filtered through the respective syringe filters (Figure 1).

Figure 1: Initial clarification and subsequent sterile filtration of cell culture supernatants under aseptic conditions using a Minisart® High Flow syringe filter with a pore size of 0.22 µm.

The mAb titer was determined in both the unharvested and the harvested cell culture fluids using the Octet QK$^e$ system equipped with a protein A Biosensor (Pro A) supplied by FortéBio, without the need for any inconvenient sample preparation. Recovery was calculated from the titers determined.

As samples with different volumes between 25 mL and 31 mL were compared, the relative mAb yield was calculated for each sample (Equation 1).

$$\frac{Volume\ of\ Filtrate\ [mL] \times mAb\ Titer\ Filtrate\ \left[\frac{mg}{mL}\right]}{Sample\ Volume\ CCF\ [mL] \times mAb\ Titer\ CCF\ \left[\frac{mg}{mL}\right]} \times 100\ \% = Yield\ [\%]$$

Equation 1: Formula for calculating the relative mAb yield [%]. This was necessary to compare the results obtained for different sample volumes ranging from 24 mL – 31 mL. CCF = cell culture fluid (= cell culture broth).

Turbidity values were measured before and after clarification using the TurbiCheck WL turbidimeter supplied by Lovibond (white light source). Afterwards, the reduction in turbidity was determined by calculation of the ratio of turbidities from harvested and unharvested samples.

## Results and Discussion

The objective of this study was to compare the suitability of two different syringe filter types for clarification of mAb supernatants with regard to particle reduction, mAb recovery, yield and usage of syringe filters.

For the experiments, we used various cultivation systems and expression vectors. With this approach, we generated a heterogeneous range of characteristics with respect to viable cell count, viability, turbidity, mAb product and titer (Table 1). In particular, the turbidity of the cell culture at harvest ranged from 457 NTU to 1431 NTU; the viable cell count, from $4 \times 10^6$ to $16 \times 10^6$ cells/mL; viability, from 48% to 89%; and mAb titers (cell cultures), between 0.2 mg/mL and 8.8 mg/mL. This diversity was the prerequisite for making an accurate statement concerning syringe filter suitability.

To determine particle reduction, we examined the turbidity of the cell culture and the filtrate. We found that both filter types removed particles efficiently from the supernatant. The filtrate of the Acrodisc® device showed an average turbidity of 17.7 NTU and that of Minisart® High Flow a mean of 17.6 NTU. When the entire clarification process is considered, i.e. centrifugation and filtration combined, this results in a relative reduction in turbidity of between 93.8% and 98.8%. Remarkably, the turbidity in the filtrate does not depend on the initial turbidity of the cell culture (Figure 2).

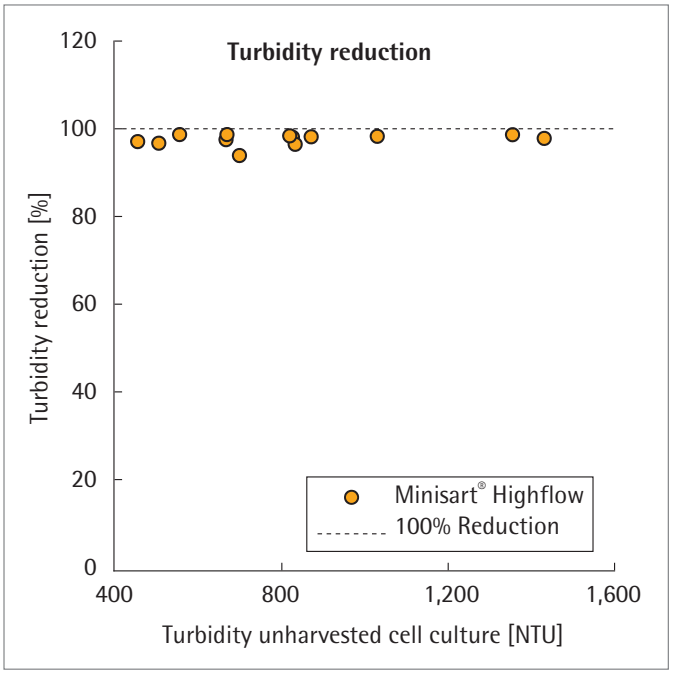


Figure 2: Turbidity reduction [%] in relation to the turbidity of the unclarified cell culture. The clarification procedure comprises a centrifugation step and a microfiltration step. The reduction in turbidity does not depend on the turbidity of the cell culture. This applies to both Minisart® High Flow and Acrodisc® (figure not shown because the data points are virtually identical).

| Expression vector No. (V) \| MAb product | Cultivation system | VCC [$10^5$ cells/mL] | Viability | Turbidity cell culture [NTU] | MAb titer cell culture [mg/mL] | Sample volume [mL] |
|---|---|---|---|---|---|---|
| V1 \| IgG1 | STR (5 L) | 86.9 | 58% | 1431 | 7.8 | 31 |
| V1 \| IgG1 | STR (5 L) | 155.2 | 78% | 1355 | 6.0 | 31 |
| V1 \| IgG1 | STR (5 L) | 163.6 | 89% | 828 | 8.8 | 31 |
| V2 \| fc fusion protein | SF (25 mL in 125 mL) | 121.0 | 71% | 1031 | 0.2 | 25 |
| V3 \| IgG1 | SF (25 mL in 125 mL) | 73.0 | 64% | 508 | 0.9 | 25 |
| V4 \| fc fusion protein | SF (25 mL in 125 mL) | 47.7 | 67% | 457 | 0.4 | 24 |
| V5 \| IgG2 | SF (25 mL in 125 mL) | 112.6 | 67% | 873 | 0.7 | 23 |
| V6 \| fc fusion protein | SF (300 mL in 1 L) | 42.2 | 69% | 701 | 1.8 | 25 |
| V6 \| fc fusion protein | SF (300 mL in 1 L) | 43.5 | 62% | 834 | 1.2 | 25 |
| V7 \| IgG2 | SF (300 mL in 1 L) | 38.3 | 48% | 821 | 0.4 | 25 |
| V8 \| IgG1 | SF (300 mL in 1 L) | 69.9 | 73% | 558 | 1.6 | 25 |
| V9 \| IgG1 | SF (300 mL in 1 L) | 52.3 | 59% | 669 | 0.3 | 25 |
| V10 \| bispecific antibody | SF (300 mL in 1 L) | 46.1 | 69% | 671 | 0.6 | 25 |

Table 1: Overview of various sample types (expression vectors | mAb products) and their parameters, such as cultivation system (STR = stirred tank reactor and SF = shake flask), viable cell count (VCC) and viability, after 14 days, as well as turbidity of the cell culture at harvest. Clarification tests were run with both syringe filters so that the respective volumes were clarified with both filter versions to obtain an objective comparison.

The various cell culture samples yielded titers of monoclonal antibodies in a range of 0.2 g/L to 8.8 g/L. The mAb titers of the filtrate ranged from 0.2 g/L to 8.2 g/L for both brands of syringe filter, resulting in recovery rates of between 89.9% and 103.9% (average: 97.7%) for Minisart® High Flow and 86.9% and 107.3% (average: 98.2%) for Acrodisc®. It should be emphasized that recovery was independent of the cell culture titer (Figure 3). This is important for regularly monitoring mAb titers during cultivation with different levels of product concentrations.

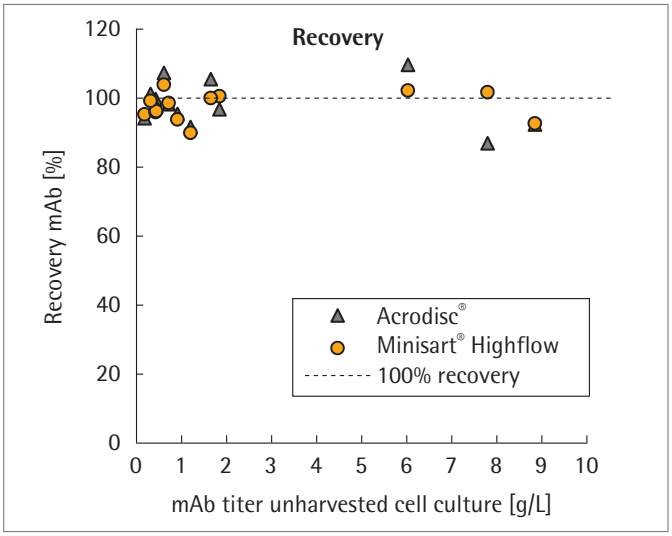


Figure 3: Recovery [%] of mAb products in relation to the mAb titer of the unclarified cell culture; this recovery was not affected by the syringe filter type and was, on average, 98.2% for Acrodisc® and 97.7% for Minisart® High Flow. No impact of the syringe filter used was observed on mAb recovery in a range of 0.3 g/L to 8.8 g/L of the cell culture titers.

The relative yield per sample was the same for both syringe filter types, despite differences in the housing design and the number of filters used per sample (Figure 4).

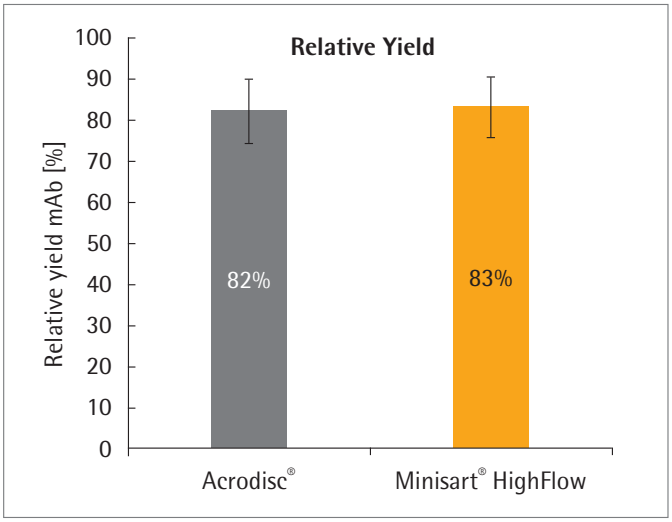


Figure 4: Average values of the relative yield [%] of various mAb products. The relative yield of a sample is the ratio of the total mAb quantity in the filtrate to that in the unharvested cell culture. As a result, we found no difference between both filter types regardless of the syringe filter design and the number of filters used.

To compare throughput and filter usage, we determined the following for each sample: volume of the supernatant, volume of the total filtrate and the required number of syringe filters used. The average throughput for Acrodisc® was 9.3 mL; for Minisart® High Flow, 18.0 mL (Figure 5). This 100% discrepancy cannot be explained by the slight difference in the effective filtration area (Acrodisc®: 5.8 cm², Minisart® High Flow: 6.2 cm²). More likely, differences in the structural design of the polyethersulfone membrane utilized in the devices could be the reason for this observation. Consequently, we found an average filter usage rate per sample of 2.5 filters for Acrodisc® and 1.4 filters for Minisart® High Flow.

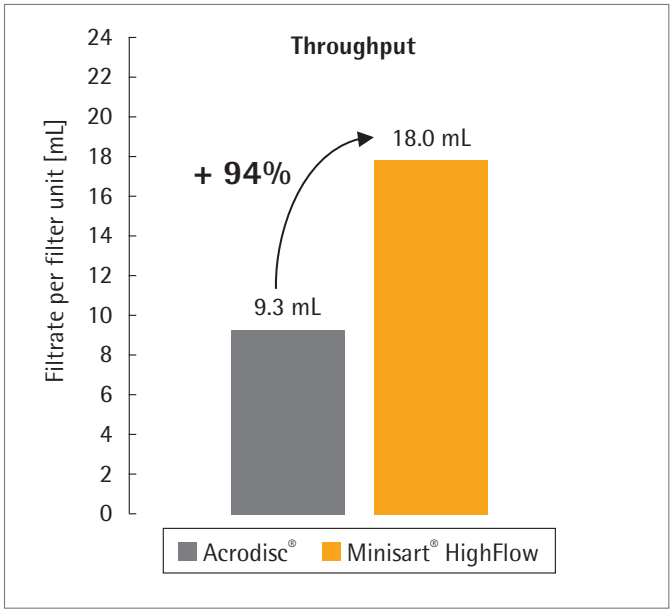


Figure 5: CHO cell culture supernatants were filtered through two different syringe filter types (0.22 µm): Sartorius Minisart® High Flow and Pall Acrodisc®. The average throughput per syringe filter was determined. Differences in throughput between both filter types were probably not caused by the difference in effective filtration area (Acrodisc®: 5.8 cm², Minisart® High Flow: 6.2 cm²), but most likely by differences in structural membrane design.

These results demonstrate that the Minisart® High Flow enables filtration of larger volumes than does Acrodisc® before clogging, while other parameters like turbidity, mAb recovery and relative yield showed the same high performance.

## Conclusion

Microfiltration is the most common, indispensable step after centrifugation of a cell culture sample. Syringe filters are often the ideal choice for processing a small number of samples with volumes of < 25 mL. Using the right filter can significantly facilitate the task and reduce the number of devices needed. In this study, we compared two filter types that had slightly different filtration areas (Pall Acrodisc®: EFA = 5.8 $cm^2$ and Sartorius Minisart® High Flow: EFA = 6.2 $cm^2$). However, no significant differences regarding turbidity, recovery or yield per sample were found. What was observed is the clear impact the filter type has on filtration performance. With its 7% larger filtration area, Minisart® High Flow achieved 94% higher filtrate volumes per device and thus enabled the number of syringe filters used per sample to be halved. This finding emphasized that the efficiency of supernatant clarification can be improved substantially without any impairment of other relevant parameters.


## Acknowledgments

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## Abbreviations

| | |
|---|---|
| CHO | Chinese hamster ovary |
| mAb | monoclonal antibody |
| EFA | effective filtration area |
| NTU | nephelometric turbidity units |




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