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Application Note

Optimizing Downstream Processing for an Inactivated Rabies Vaccine

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Objective

While rabies is a preventable and curable disease, it continues to have an impact on human and animal health in developing countries. According to the World Health Organization (WHO), infection causes tens of thousands of deaths every year, mainly in Asia and Africa (1). Effective vaccines do exist, but they are not always readily available or accessible to those in need.

Optimizing virus yield during the manufacturing process is essential for development of a low-cost rabies vaccine, which would help ease the burden in low and middle income countries.

We are collaborating with the Institut Pasteur de Tunis, one of the main research centers in Tunisia with vaccine manufacturing capabilities, to optimize their Rabies vaccine process. The overall goal is to establish a rapid, scalable and GMP compliant process for more cost-effective production and increased yield. This white paper describes the improvements made to the downstream portion of the process, specifically, use of single-use technologies and novel techniques for clarification and ultrafiltration/ diafiltration.

Summary of Results

- The combination of Millistak+® HC C0HC depth filters and a Polysep™ prefilter used prior to tangential flow filtration improved overall yield of the clarification step from approximately 50% to more than 90%
- High loading achieved on the clarification step enabled cost-efficient and low footprint scale-up
- Pellicon® tangential flow filters allowed concentration and diafiltration of the rabies viral vaccine with minimal loss

Current Process Challenges

The upstream portion of the process starts with production of virus in Vero cells grown on microcarriers in perfusion mode. The upstream process had been optimized in a bench-top bioreactor and then further scaled to a 20L bioreactor (12L working volume) with a maximal cell viability of 81% and a cell density that can reach 9.10^6 cells/mL (2).

Prior to optimizing the downstream process, viral harvests obtained through the culture were clarified using an 8 µm cellulosic membrane followed by a 0.45 µm PVDF membrane (**Figure 1**). Inactivation using β-propiolactone (BPL) and sucrose stabilization was performed prior to filtration on the 0.45 µm membrane. Zonal centrifugation in a sucrose density gradient was then used for purification.

Yield losses observed with an older, disc-style 0.45 µm membrane filter that was not scalable drove interest in integration of new clarification technologies to minimize this loss, enable scaling and modernize the process. The downstream process was further optimized downstream with the addition of tangential flow filtration (TFF) which allows concentration of the filtrate and buffer exchange prior to the chromatography step.

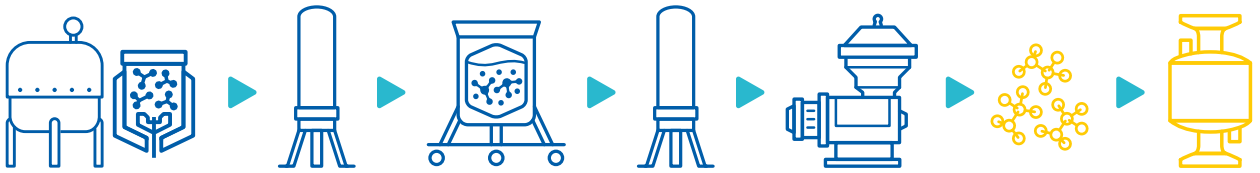

Figure 1. Initial rabies vaccine production process.

Table 1 summarizes the depth filters evaluated for clarification, prefilters and TFF membranes that were evaluated for use in downstream processing of the rabies vaccine. The Clarisolve® 60HX filter is made of polypropylene and is not charged. The prefilters are 0.5 µm rated with different chemistries; Polysep™ is a mix of cellulose and glass fiber, Durapore® is polyvinylidene fluoride (PVDF) and Millistak+® is cellulose and diatomaceous earth.

Prefilter	Details	Membrane Area (cm^2)	Catalog Number	
Polysep™ 1.0/0.5 µm	Prefilter: BGF Filter: MCE	17.7	SGW6A47FF3	
Milligard® 1.2/0.5 µm	MCE	17.7	SWSCA47FF3	
Durapore® 0.45 µm	PVDF	3.5	SPHLA25NB6	
Depth Filter	**Details**	**Membrane Area (cm^2)**	**Catalog Number**	
Millistak+® D0HC	25CE + 40DE	23	MD0HC23CL3	
Millistak+® C0HC	30DE + 60DE	23	MC0HC23CL3	
Clarisolve® 60HX	Polypropylene	23	CS60HX01L3	
TFF Device	**Membrane**	**Area (m^2)**	**Catalog Number**	**Screen**
Pellicon® 2 cassette	Biomax® 100 kDa	0.1	P2B100V01	V
Pellicon® 2 cassette	Biomax® 300 kDa	0.1	P2B100C01	C

Table 1. Specifications of the depth filters, pre-filters and TFF membranes evaluated for optimizing downstream processing.

Optimizing the Downstream Process: Clarification

The bioreactor used in the original process included a stainless-steel spin filter which was used to remove microcarriers from the feed stream. In parallel with this study to optimize the downstream process, a Clarisolve® 60HX depth filter was evaluated for the removal of microcarriers as a potential replacement of the currently used stainless steel spin filter (data not shown). While the concentration of microcarriers used in culture was approximately 3 g/L, the ability of Clarisolve® depth filter to remove carriers at a concentration up to 6 g/L was demonstrated. Additional characteristics of the feed streams are detailed in **Table 2**.

Product of Interest	Rabies Vaccine
Size (nm)	180 nm long and 75 nm wide
Product concentration (mg/L)	2.27
Other components	VP-SFM medium + Sucrose 5%
Cell line type	Vero. Cytodex 1 microcarrier (3g/L)
Cell density (x10^6 cells/mL)	3 to 4
Cell viability (%)	<30%
Fermentation type	Perfusion
pH	7.4
Stability	Several days at +4° C
Phase	Preclinical

Table 2. Characteristics of the virus production feed stream.

The impact of different hydraulic conditions in combination with Millistak+® D0HC and C0HC depth filters on yield are shown in **Table 3**. When the flux was increased across the C0HC filter (comparing trials Millistak+® C0HC #1 and Millistak+® C0HC #2), there was an increase in yield from 52% to 92%. Addition of a buffer flush step to trials Millistak+® D0HC and Millistak+® C0HC improved recovery of the virus (**Figure 2**).

Depth Filter	Trial Flux (LHM)	Trial Loading (L/m^2)	Trial Endpoint Pressure (psi)	Initial Turbidity (NTU)	Filtrate Pool Turbidity (NTU)	Yield (%)
Millistak+® C0HC #1	103.2	369.6	0	1.31	0.7	52
Millistak+® C0HC #2	181.2	347.8	0	5.45	1.66	92

Table 3. Summary of results using Millistak+® C0HC depth filters.

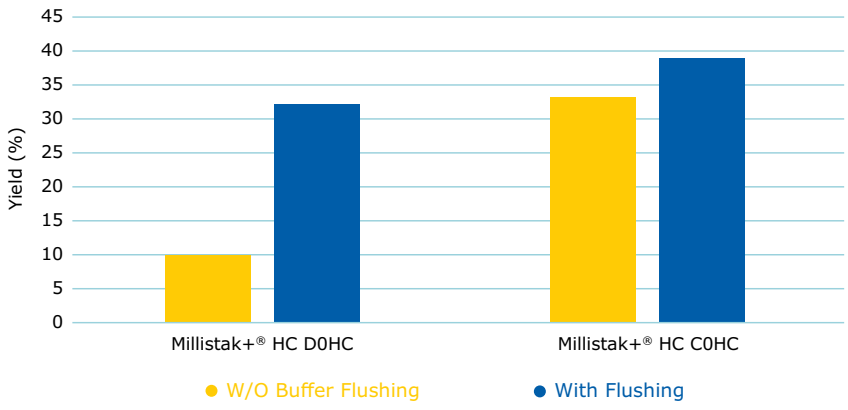

Figure 2. Use of a buffer flush step improved recovery of the virus.

Following these initial results, a larger feed stream volume was clarified using the Millistak+® C0HC filter and Polysep II™ 1.0/0.5 µm pre-filter. The ability to filter a larger volume using the same filter footprint provides the opportunity to improve process economics.

This trial demonstrated the impact of loading on the recovery. Two hold-up volumes of phosphate buffered saline (PBS) buffer were added following depth filtration. The final yield could be significantly improved from 39% to 91% when the filtration loading was increased from 400L/m² to more than 1700L/m² (**Figure 3, Table 4**). This result can be explained by saturation of non-specific adsorption sites of the filter.

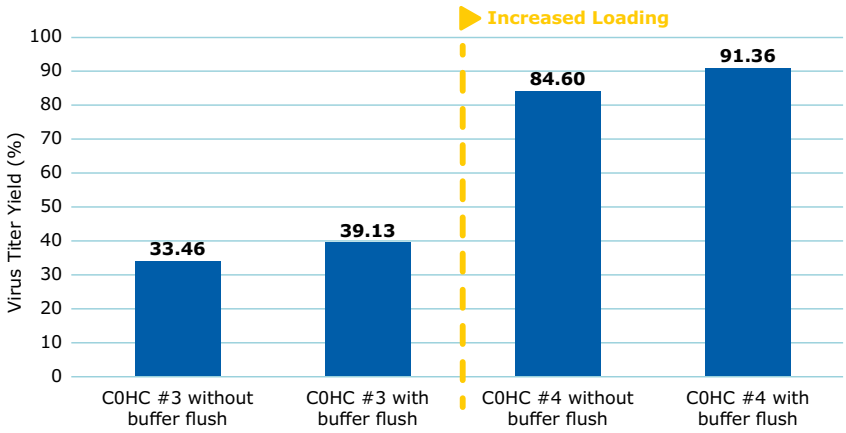

Figure 3. Impact of increased loading on yield.

Depth Filter	Trial Flux (LHM)	Trial Loading (L/m²)	Trial Endpoint Pressure (psi)	Initial Turbidity (NTU)	Filtrate Pool Turbidity (NTU)	Yield (%)
Millistak+® C0HC #4	134.1	1739.1	0	5.3	~2	91.4
Polysep® II 1.0/0.5 µm filter	166.6	434.8	0			

Table 4. Results of loading optimization study.

Optimizing the Downstream Process: Concentration/Diafiltration

Pellicon® 2 TFF devices with 100 kDa and 300 kDa BioMax® membranes were tested at 4 L/min/m². The optimization curves (data not shown) indicated an optimal transmembrane pressure (TMP) of 20 and 30 psi respectively. Both membranes provided similar yields as shown in **Table 5**.

TFF Membrane	CF/DF	Trial Loading (L/m²)	TMP (psi)	Conc. Flux (LMH)	Diaf. Flux (LMH)	Yield (%)
300 kDa	13.1/5.9	18.47	30	185	142	85.0
100 kDa #1	11.7/5.4	18.3	20	143	123	88.4
100 kDa #2	11.7/5.1	58.95	18	130	93	79.1

Table 5. Comparison of 300 kDa and 100 kDa membranes for concentration/diafiltration.

Figures 4 and 5 show the impact of the volumetric concentration factor (VCF) and diavolumes on performance of the 100 kDa BioMax® TFF membranes, respectively. In both cases, permeate flux, TMP and Pin (Inlet Pressure) remained within the desired ranges.

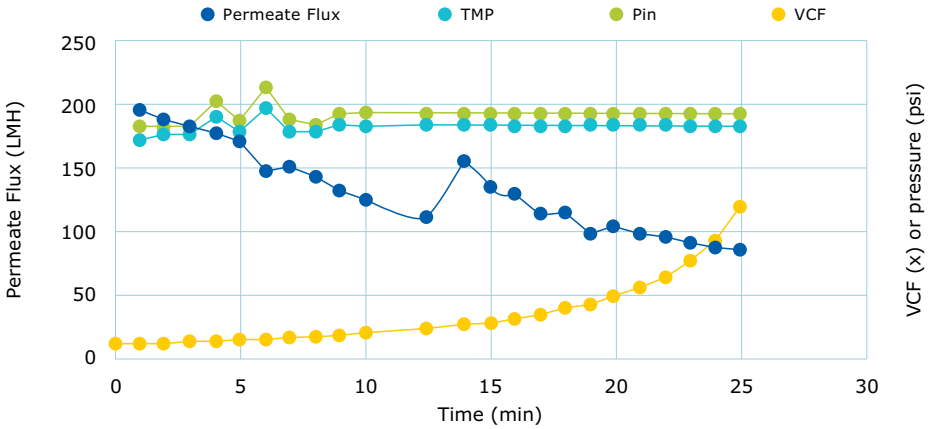

Figure 4. Impact of VCF on 100 kDa BioMax® TFF membrane operating parameters.

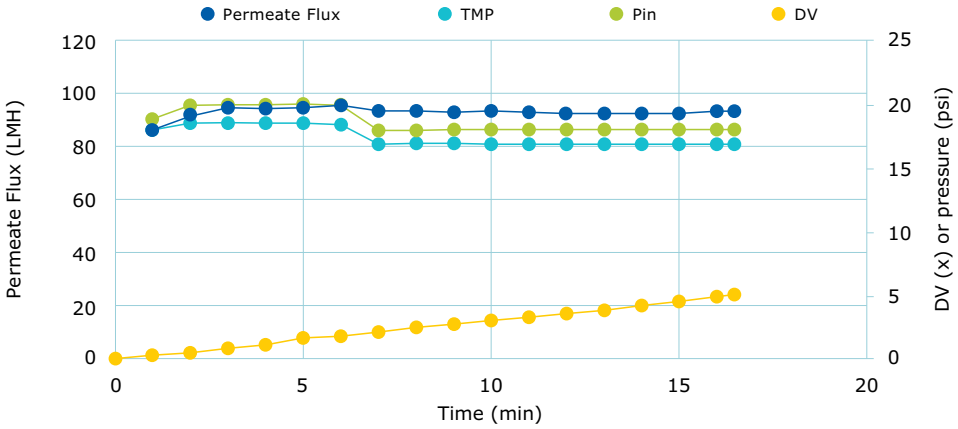

Figure 5. Impact of diavolumes on 100 kDa BioMax® TFF membrane operating parameters.

Samples from permeate of the ultrafiltration and diafiltration steps were analyzed by SDS-PAGE (**Figure 6**). No bands were detected indicating that there was no virus present in the permeate samples; the amount was also estimated by ELISA to be 0 µg/mL (data not shown).

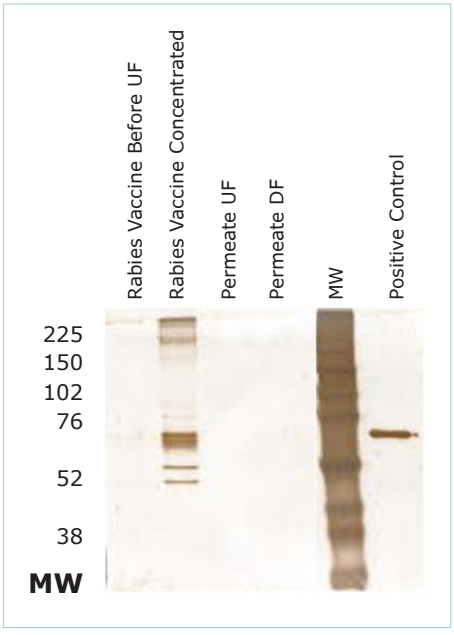

Figure 6. Silver staining analysis of samples following ultrafiltration and diafiltration.

Conclusion

The need for a robust, cost-effective process for the manufacture of a rabies vaccine is clear.

In this study, we collaborated with the Institut Pasteur de Tunis to optimize the clarification step and integrate TFF into the downstream process of their virus manufacturing process. The combination of Millistak+® HC C0HC depth filters with a Polysep™ II prefilter resulted in a significant improvement to the overall yield of the clarification step. Yield was increased with higher loading on the filter and addition of a post-filtration buffer flush. The high loading achieved on the clarification step enables a more cost-efficient and smaller footprint scale-up – essential for the success of rabies vaccine manufacturing. In addition, Pellicon® 2 100 kDa and 300 kDa filters performed with no noticeable issue, allowing the concentration and diafiltration of the rabies viral vaccine with minimal loss.

The potential benefits incorporating this updated process include a significantly increased yield and loading in robust clarification step leading to a lower footprint and lower cost of goods, a bioburden reduction step: changing an outdated disc-format with limited loading resulting with yield and long filtration time to scalable and high performing capsule format, and a reproducible, scalable and easy-to-operate process (TFF versus Ultracentrifugation). Further experiments are ongoing to improve the chromatography step: another critical unit operation in the purification process of the vaccine.

References

1. https://www.who.int/news-room/fact-sheets/detail/rabies
2. Trabelsi et al. 2005; 2006

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