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White Paper

Filtration strategies for optimal development and purification of a Foot and Mouth Disease virus produced in BHK21 cells

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Foot-and-mouth disease (FMD) is a highly contagious and sometimes fatal viral disease that affects cloven-hoofed animals. Massive vaccination with at least 80% coverage is one of the control strategies implemented to prevent virus introduction and development. FMD vaccine manufacturing follows a simple multi-step process, a general outline of which is provided in **Figure 1.**

We collaborated with MEVAC to optimize upstream and downstream processes for FMD vaccine manufacturing to establish a scalable, cost-efficient and GMP compliant process. This white paper focuses on the integration of new filtration strategies in both upstream and downstream processes.

Objective

Optimize the filtration strategies used during downstream processing of foot and mouth disease (FMD) virus vaccine to ensure cost effective and robust manufacturing

Collaborator

MEVAC (Middle East for Vaccines) is a private company in Egypt for development and manufacturing of vaccines

Results

- Cellvento® BHK-200 serum-free medium for FMDV production in BHK21 cells can easily be filtered using Millipore Express® filters for bacteria or and/or mycoplasma clearance
- High capacity and low turbidity achieved on the clarification step allowing a very cost-efficient and low footprint scale-up with the Millistak+® HC C0HC filter
- Pellicon® 2 300 kDa or 1000 kDa tangential flow filtration can be implemented for the concentration and diafiltration of the FMD vaccine
- The adjuvant filtration process which had required three different filtration steps and a total of eleven 30” filters was replaced by a single Millipore Express® PHF 0.2 µm filter

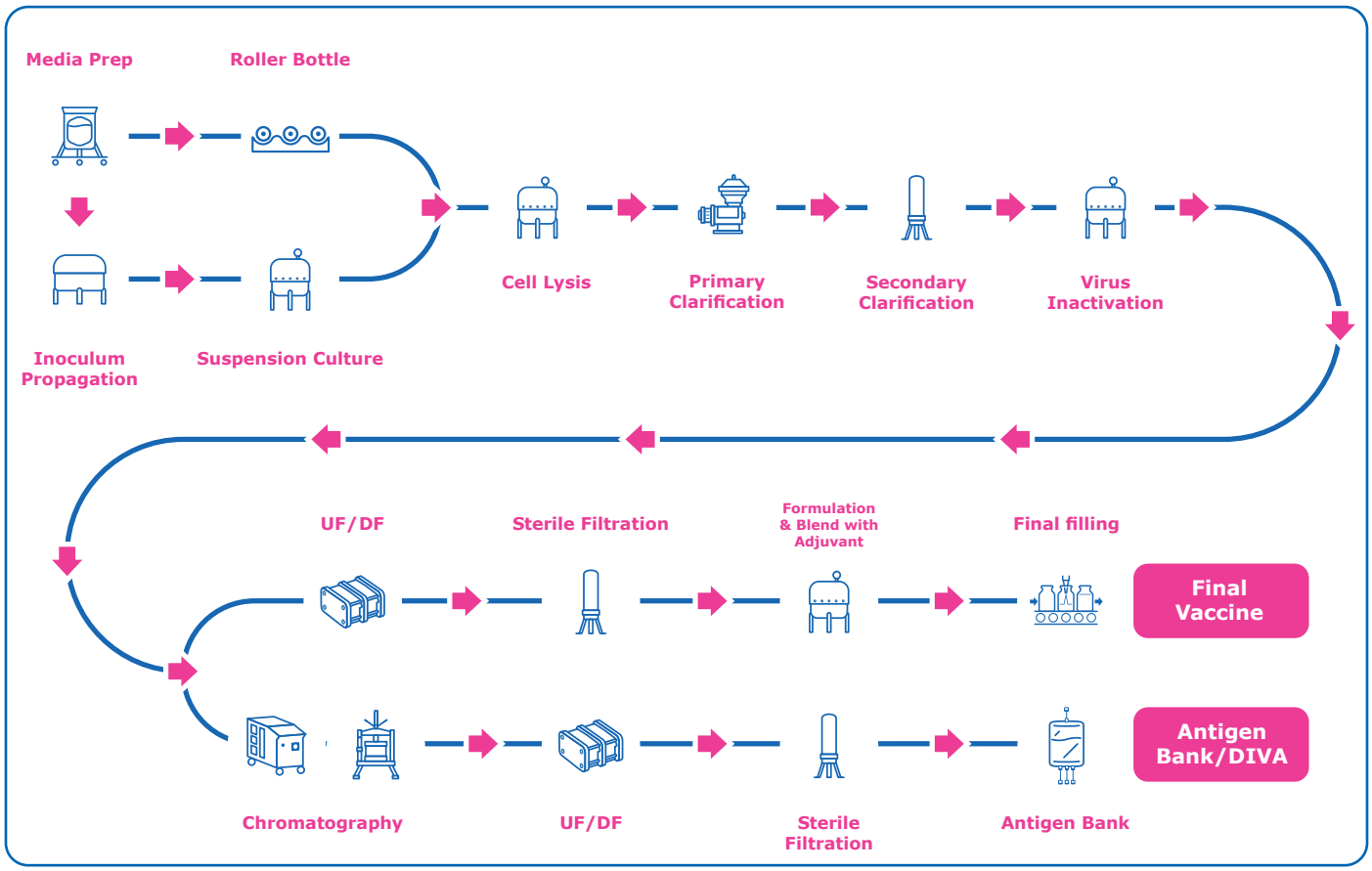

Figure 1. Typical process for manufacturing FMD vaccines.

Optimizing Media Filtration

BHK21 cells grown in suspension are typically used for production of FMD vaccines. This cell line is banked to grow in Glasgow Minimum Essential Medium (GMEM) supplemented with 5% tryptose phosphate broth (TPB) and 10% serum in spinner flasks, roller bottles, or small bioreactors.

To eliminate the risk presented by bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE) agents and mycoplasma contaminations, Cellvento® BHK-200 serum free medium was used in this process. The medium is formulated without animal derived components and optimized for the culture of suspension BHK21 cells at high-density and viability with efficient propagation of viruses. Cells grown in Cellvento® BHK200 medium are first adapted via serial passaging in the medium supplemented with decreasing concentration of serum. Adapted BHK21 cells can then be grown as suspension cultures in T-flasks, shaker flasks, spinner bottles or stirred tank bioreactors with a higher growth in comparison to GMEM/TPB/serum cultures.

During preparation of the media, sterilizing-grade filtration was performed, and different filters were screened in order to define the most efficient option (**Table 1**).

Table 1. Several sterilizing-grade filters were evaluated for media preparation.

Filter	Filter details	Cat. No	Membrane area (cm²)
Durapore® 0.22 µm	PVDF (bacterial retention)	**SVGLA25NB6**	3.5
Millipore Express® SHF 0.2 µm	PES (bacterial retention)	**SGEPA25NB6**	3.5
Millipore Express® SHC 0.5/0.2 µm	PES (bacterial retention)	**SHGEA25NB6**	3.5
Millipore Express® SHR 0.1 µm	PES (bacterial, mycoplasma retention)	**SVEPA25NB6**	3.5
Millipore Express® SHR-P 0.5/0.1 µm	PES (bacterial, mycoplasma retention)	**SHVEA25NB6**	3.5

BHK200 medium was challenged against different sterilizing-grade filters for bacterial and/or mycoplasma retention **(Table 2)**. The best capacity was observed with the Millipore Express® SHF 0.2 µm and SHC 0.5/0.2 µm filters for the sterilizing-grade options; the SHC 0.5/0.2 µm filters showed no signs of plugging in comparison to the single layer Millipore Express® filter. Millipore Express® SHC and SHR-P filters have an on-board polyethersulfone membrane prefilter which protects the 0.2 or 0.1 µm membrane, respectively, from premature plugging. Similar performances were observed for the two mycoplasma-retentive Millipore Express filters. The highest Vmax™ was obtained using the SHC 0.5/0.2 µm filter.

Figure 2 shows the throughput profile over time for the five filters evaluated. A comparison of the Millipore Express® SHF 0.2 µm and SHC 0.5/0.2 µm filters showed that the single membrane provided a high flux while addition of the prefilter provides high capacity.

Table 3 summarizes filter sizing recommendations for processing 1000 L of medium and indicates that the Millipore Express® SHC 0.5/0.2 µm filter would enable a smaller footprint.

Table 2. Filtration trial results.

Prefilter	Diff. Pressure	Trial Loading	Trial Flux Decay	Vmax
	psi	L/m²	%	L/m²
Durapore® 0.22 µm	10	540.0	69.6	1782.09
Millipore Express® SHF 0.2 µm		1437.1	71.6	2080.43
Millipore Express® SHC 0.5/0.2 µm		1260.0	2.5	1758003.38
Millipore Express® SHR 0.1 µm		888.6	36.8	N/A
Millipore Express® SHR-P 0.5/0.1 µm		965.7	35.8	2685.21

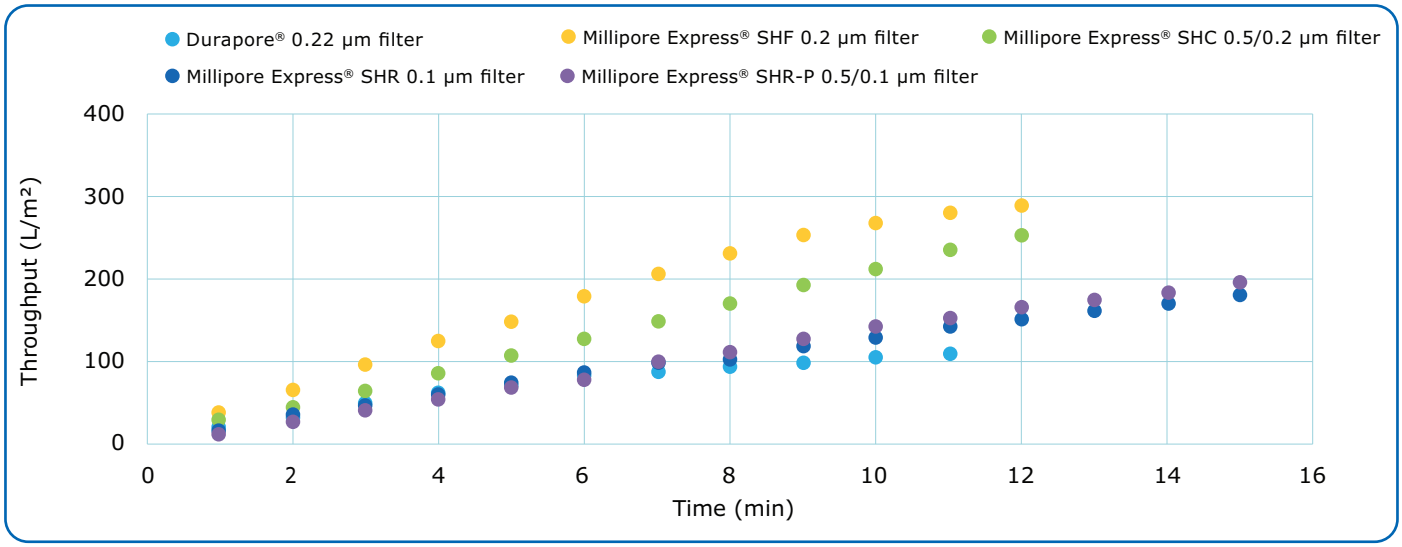

Figure 2. Throughput profile over time.

Table 3. Sizing recommendations for processing 1000 L of medium.

Filter	Batch Volume	Process Loading	A_{min}[1]	Recommended Configuration	Resultant Area	Final Safety Factor
	L	L/m²	m²		m²	x
Durapore 0.22 µm	1000	724.6	0.71	1x OptiCap XL20	1.38	1.9
Millipore Express® SHF or PHF 0.2 µm		925.9	0.48	1x OptiCap XL20	1.08	2.2
Millipore Express® SHC 0.5/0.2 µm		7692.3	0.08	1x OptiCap XL3	0.13	1.6
Millipore Express® SHR 0.1 µm		1666.7	0.32	1x OptiCap XL10	0.60	1.9
Millipore Express® SHR-P 0.5/0.1 µm		1020.4	0.38	1x OptiCap XL20	0.98	2.6

1_{Amin} is the minimum calculated filter area to achieve filtration (with no safety factor)

Optimizing Clarification

The objective of clarification is to remove cell debris and contaminants and recover virus. Zonal centrifugation is commonly used for finally clarification while others use a body feed sparkler assembly. Since solid content in viral vaccine harvest is low, depth filters or disc stack centrifuge typically work well for primary clarification.

Two harvests were tested for clarification or with a Millistak+® HC C0HC depth filter using the pressure max and turbidity max (Pmax/Tmax) methodology at a constant flow rate; two prefilters were also evaluated **(Table 4)**. The results shown in **Figures 3** and **4** indicate that the Millistak+® HC C0HC filter was capable of processing the BHK21 cell culture in a single step, without requiring a secondary depth filter. The pressure rise occurred at 180 L/m² and turbidity remained below 15 NTU. **Table 5** summarizes the Millistak+® HC C0HC filtration results.

Table 4. Specification of the depth filter and prefilters evaluated in the study.

Filter	Filter details	Membrane area (cm²)	Cat. No
Millistak+® HC C0HC	30DE + 60DE	23	**MC0HC23CL3**
Durapore® 0.45 µm	PVDF, Bioburden Reduction	3.5	**SPHLA25NB6**
Milligard® PES 1.2/0.45 µm	PES, Bioburden Reduction	3.5	**SMP4A25NB6**

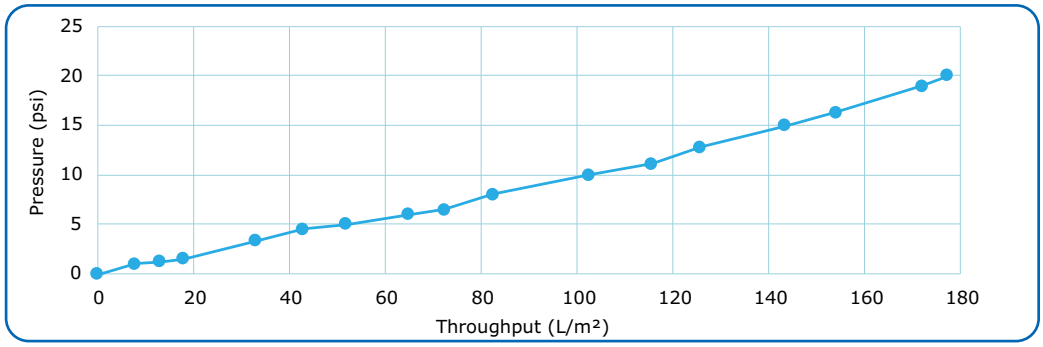

Figure 3. Millistak+® HC C0HC pressure profile.

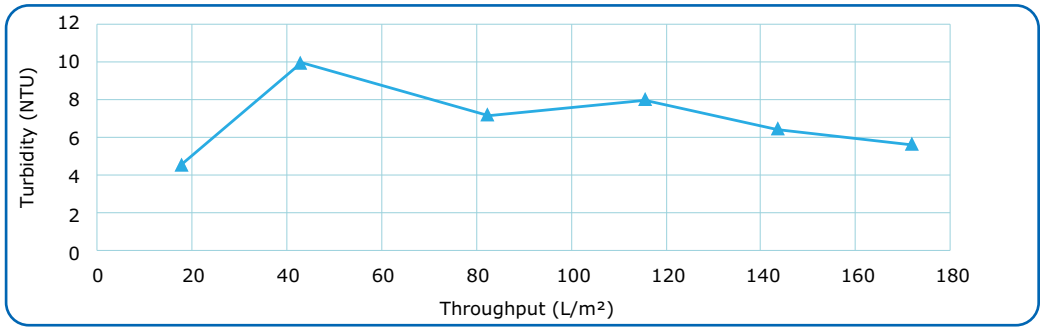

Figure 4. Millistak+® HC C0HC turbidity profile.

Table 5. Summary of Millistak+® HC C0HC filtration results.

Trial flux (LMH)	Trial loading (L/m²)	Trial endpoint inlet pressure (psi)	Harvest turbidity (NTU)	Filtrate pool turbidity (NTU)
152	177.4	20 (Pmax)	260	9.5

Filterability of the clarified harvest was also assessed on bioburden reduction membranes. Constant pressure tests were performed at 10 psi with Durapore® 0.45 µm and Milligard® PES 1.2/0.45 µm small scale filters to determine the theoretical maximum volume (Vmax™) of each solution filterable on the membrane. The initial filtrate flux (Qi) was also determined to estimate the minimum area required to filter the batch.

As shown in **Table 6**, the Milligard® PES 1.2/0.45 µm filter showed better performance than the Durapore® 0.45 µm filter and would be the preferred option for the bioburden reduction step, prior to concentration and diafiltration of the product. **Table 7** summarizes filter sizing recommendations for processing 1000 L of medium.

Table 6. Vmax bioburden reduction experiments.

Membrane filter	Membrane filter loading (L/m²)	Trial Flux decay (%)	Sterilizing-grade filter Vmax (L/m²)	Qi (LMH)
Durapore® 0.45 µm	478.9	98	545.2	23716.7
Milligard® PES 1.2 /0.45 µm	596.0	54	1667.2	25671.9

Table 7. Sizing recommendations for processing 1000 L of harvest

Filter	Amin (m²)	Suggested config.	Area (m²)	Safety Factor	Processloading (L/m²)
Millistak+® HC C0HC	5.3	7x 1.1 m²	7.7	1.5	130.0
Durapore® 0.45 µm filter	1.84	3x Opticap XL20	3.7	2.0	268.8
Or Milligard® PES 1.2/0.45 µm filter	0.61	1x Opticap XL20	1.2	2.0	833.3

Optimizing Concentration/Diafiltration

Tangential flow filtration (TFF) is commonly used to remove inactivating agent by diafiltration and concentration of virus.

The concentration step of the FMD vaccine production process was optimized using Biomax® membranes in a Pellicon® 2 cassette for TFF **(Table 8)**. These membranes are made of polyethersulfone which is designed to reduce non-specific protein binding and are resistant to harsh chemicals used in cleaning, biological decontamination and sanitization. Membranes are available in four screen formats: V screen (suspended), C screen (coarse), A screen (fine) and D screen (for high viscosity).

Table 8. Specifications of ultrafiltration membranes tested

Device	Membrane	Screen	Cat. No.	Area (m²)
Pellicon® 2 cassette	Biomax®300 kDa	C	**P2B300C01**	0.1
	Biomax®1000 kDa		**P2B01MC01**	

No pressure instability was observed with increasing permeate fluxes. Using the Biomax® 300 kDa membrane, the trial was stopped at 67.2 liter/m²/h LMH with a final transmembrane pressure (TMP) of 5.9 psi with the permeate valve completely open **(Figure 5)**. The starting TMP was 3.96 psi meaning that the trial was stopped with a 1.5x increase in pressure. For the 1000 kDa membrane, a 1.37x increase in pressure was noted at the end of the optimization study, with a permeate valve completely open. It is typical to consider pressure instability when the increase in pressure is > 1.5-2.0 and recommended to use the membranes at 75% of the maximal working flux.

Both membranes can be used with approximately the same hydraulic performances. The 300 kDa membrane tested was new while the 1000 kDa had been previously used by MEVAC for concentration without controlled monitoring of the system pressure and flowrates (feed and permeate). Performance of the 1000 kDa membrane during this optimization study can therefore be affected by previous use and membrane polarization was unbalanced.

Recommended operating parameters for the TFF step are provided in **Table 9**.

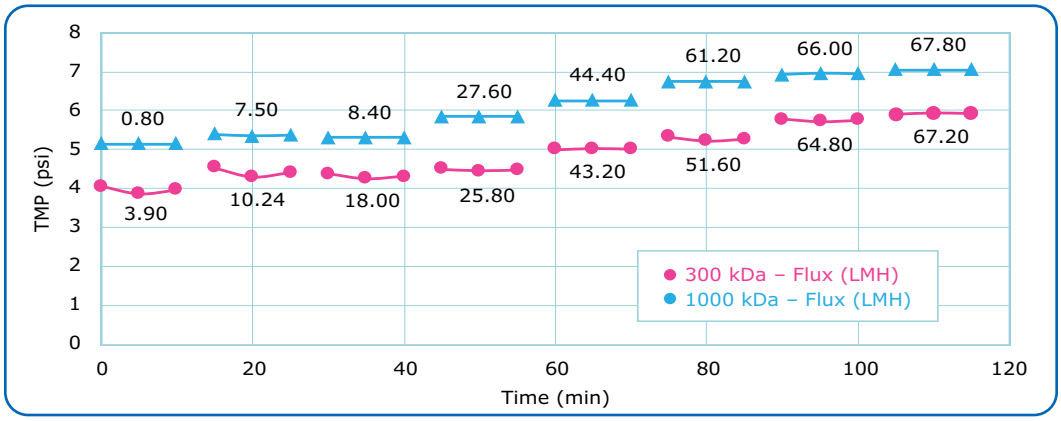

Figure 5. Results of the flux excursion study.

Table 9. Recommended operating parameters.

Membrane	Pump Flowrate	TMP (psi)	Set permeate Flux (LMH)
300 kDa	4 LMM	5	50
1000 kDa		6.5	51

Optimizing Adjuvant Filtration

Adjuvants are added to vaccine formulations to enhance the immune response and increase the level and duration of protection that is induced. Among the most used adjuvants are aluminum-based or water-in-oil or water-in-oil-in-water emulsions and lipids. Because they will be present in the final formulation, adjuvants must be sterile filtered. While it is impossible to filter an aluminum solution through a 0.22 µm sterilizing-grade filter, sterility is typically achieved through heat sterilization; sterile filtration is challenging for oil adjuvants and lipid-based formulations.

Filtration performance is affected by processing conditions, filter selection and feed stream properties. As such, careful optimization of temperature pressure, membrane and particle size and loading is essential to establish an efficient process and assure sterility.

The initial process used for sterilizing-grade filtration of FMD vaccine adjuvants included three different steps using a total of eleven 30" filters; processing time exceeded 8 hours for a 2500L batch **(Figure 6)**.

Three filters were evaluated for the ability to filter the FMD vaccine adjuvant; the best capacity was observed with the Millipore Express® PHF 0.2 µm **(Figure 6)**. Implementation of this solution allowed compression of the adjuvant filtration train to a single 30" filter **(Figure 7)**.

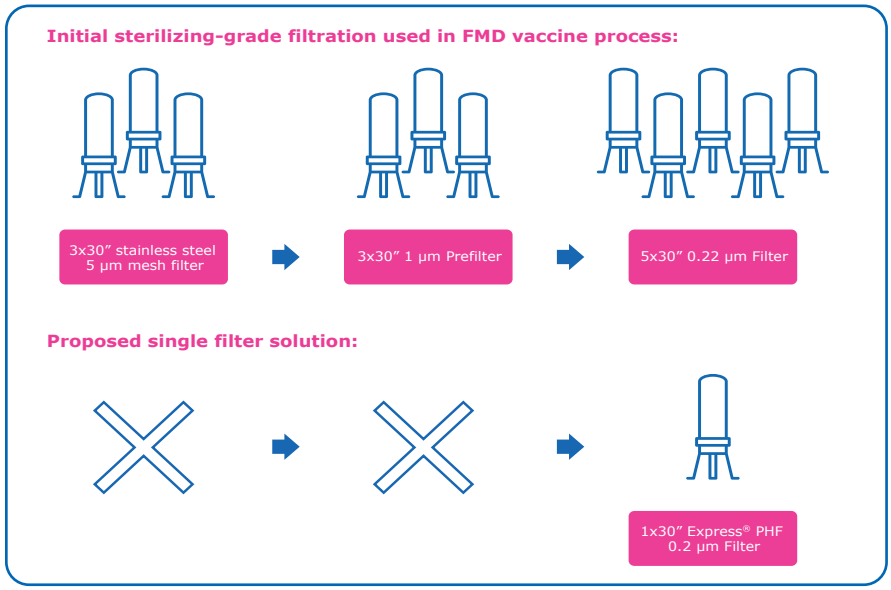

Figure 6. Initial sterilizing-grade filtration of the adjuvant used in the FMD vaccine process (A) and the new process requiring one step (B).

Table 10. FMD vaccine adjuvant filtration test results: filter details

Filter	Filter details
Durapore® 0.22 µm hydrophobic	PVDF (bacterial retention)
Millipore Express® PHF 0.2 µm	PES (bacterial retention)
Millipore Express® SHC 0.5/0.2 µm	PES (bacterial retention)

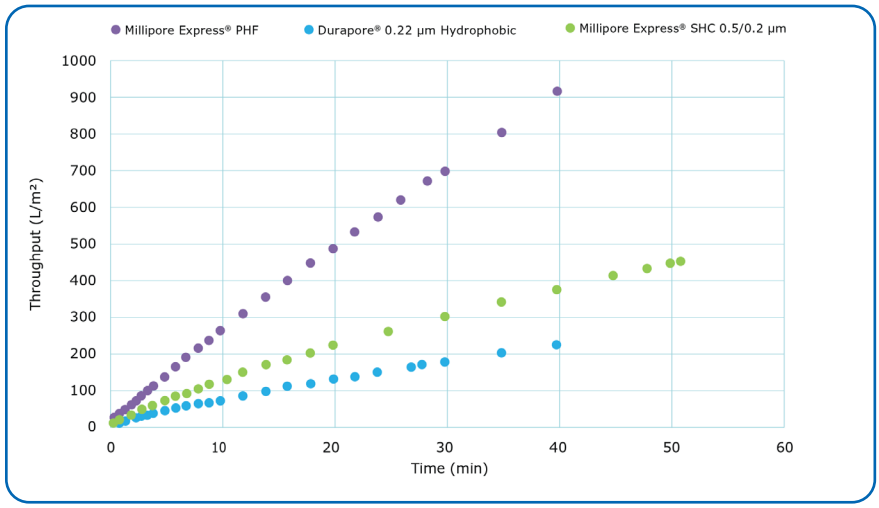

Figure 7. FMD vaccine adjuvant filtration test results

Conclusion

Incorporation of new filtration strategies delivered significant improvement to both upstream and downstream processes including media preparation, clarification, TFF and adjuvant filtration for production and purification of an FMD vaccine.

The Cellvento® BHK-200 serum free medium was easily filtered using Millipore Express® filters. The high capacity and low turbidity achieved for the clarification step allowed for a cost-efficient and low footprint scale-up using a Millistak+® HC C0HC filter while either the Pellicon® 2 300 kDa or 1000 kDa cassettes were implemented for concentration and diafiltration of the FMD vaccine. Use of a Millipore Express® PHF 0.2 µm filter allowed compression of the adjuvant filtration train from eleven to one 30”.

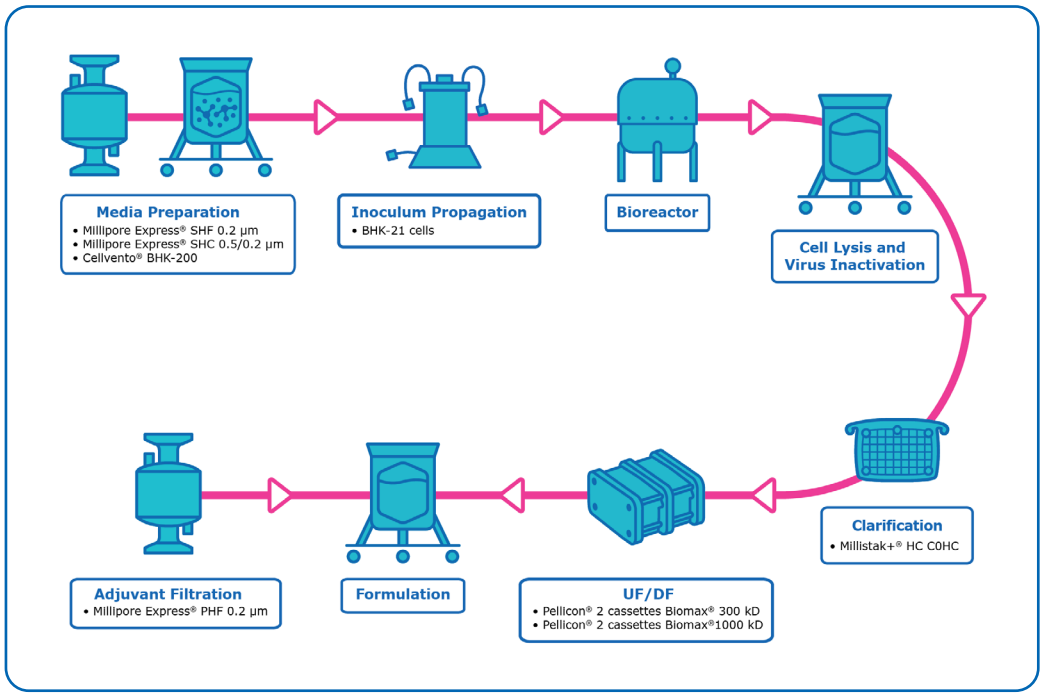

Figure 8. FMD vaccine production process

Considering the strategy to limit the impact of FMD through vaccination, and to ensure sufficient supply of vaccines, a partnership between manufacturers and process solution providers is key to enable a fast and efficient journey to commercialization. This collaboration has here enabled the implementation of a an optimized and scalable production platform; starting from the upstream with the use of a recent cell culture media and specifically adapted cells allowing the replication of the FMD virus in a completely serum-free environment. This change strongly reduced the burden on the downstream purification steps and allowed the development of high performing filtration purification strategies to develop a scalable, cost-efficient and regulatory compliant FMD vaccine production. Implementing a more robust process with improved economics is an important step towards enabling greater access to a much-needed vaccine in the animal health market.

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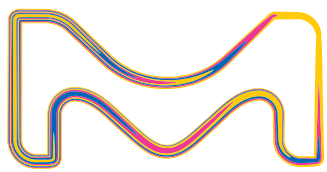

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