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White Paper

Neglected Tropical Diseases — Improving the Manufacturing Paradigm for a Novel Recombinant Protein Vaccine

Working in close partnership, Texas Children's Hospital Center for Vaccine Development at Baylor College of Medicine and Merck are combining their bioprocess and engineering expertise to advance and optimize the manufacturing process for a promising new preventive recombinant protein-based vaccine, to end the scourge of Schistosomiasis, one of the world's most devastating and pervasive parasitic infections and neglected tropical diseases.

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Introduction

Schistosomiasis is a chronic parasitic infection that ranks second only to malaria on the list of the world's most devastating infectious diseases, in terms of its public health and socioeconomic impact, according to the World Health Organization (WHO; Geneva, Switzerland). At any time, this neglected tropical disease affects an estimated 200 million people in the world's most poverty-stricken regions, with 280,000 people dying each year from Schistosomiasis-related health issues.

Among its major effects, Schistosomiasis causes chronic developmental disabilities, cognitive delays in children, severe end organ damage to the intestines, liver, and urinary tract in adolescents adults, as well as bladder cancer. In girls and women, genital Schistosomiasis may represent the most common gynecologic condition on the African continent, where it is also a leading co-factor in its HIV/AIDS epidemic.

Schistosomiasis is most prevalent across sub-Saharan Africa and the Middle East, Brazil and Venezuela in Latin America, and Philippines and China in East Asia. Schistosomiasis transmission has been reported in 78 countries. People living in areas of extreme poverty are exposed to, and infected by, the parasitic flatworms in their infective stages during routine agricultural, domestic, occupational and recreational activities involving freshwater lakes and rivers, making it impossible to break the cycle of ongoing transmission and reinfection, even for those who have been treated for the infection. (for more, see Box1, Schistosomiasis: A Primer, on page 2). It's notable that Schistosomiasis was recently noted to have emerged in Corsica, France[1], possibly as a consequence of human migrations from Africa or climate change. There are concerns that through climate change, additional sites for schistosomiasis emergence are possible[2].

Schistosomiasis: A primer

Schistosomiasis, also known as bilharzia, is caused by one of six different species of parasitic trematode worm Schistosoma, with the majority of the cases either infected with *Schistosoma mansoni (approximately one third of the global human cases), Schistosoma haematobium (two thirds), with one percent of individuals affected by Asian schistosomiasis caused by Schistosoma japonicum or Schistosoma mekongi* (Figure 1).

People become infected when they come in contact with the larval forms of these parasitic flatworms, which reproduce asexually in freshwater snails and are eventually released into the water in large numbers as infective larval schistosomes (called cercariae). These schistosomes swim freely in lakes and rivers and burrow into the skin of a human host in lakes and rivers, migrating through the blood stream and lungs to infect various organs, where it matures into another stage (schistosomula).

The cycle of transmission continues when people suffering from Schistosomiasis defecate or urinate into freshwater bodies, and the eggs are returned to the water, to be taken up by freshwater snails and later released into the water as parasitic larvae that wait for their next human host.

Long-term impact

Worm infestation can take a urogenital form damaging the kidneys, bladder, and female genital tract (from S. haematobium), or an . intestinal form (caused by *S. mansoni* and the other species of Schistosoma worm), damaging the liver and spleen over time. *S. haematobium* is also a recognized carcinogen leading to bladder cancer in Africa and the Middle East[13].

Chronic schistosomiasis is linked with numerous disease sequalae (TABLE), especially in children and adolescents. The growing burden in human hosts leads to inflammation, granulomas and host fibrosis, immune reactions and progressive organ damage, causing malaise, lethargy and failure to thrive (including both physical and cognitive deficits). Schistosomiasis is a leading cause of pediatric intellectual and cognitive deficits[14], especially in Africa, the Middle East, and Brazil

Schistosomiasis also inflicts great social stigma (particularly on women), and the worm burden is thought to compromise the immune system, making the host more susceptible to other infections. According to WHO, Schistosomiasis is thought to be an important co-factor in the spread of HIV/AIDS in developing nations.

Major clinical sequalae of Schistosomiasis in Africa, Middle East and Latin America

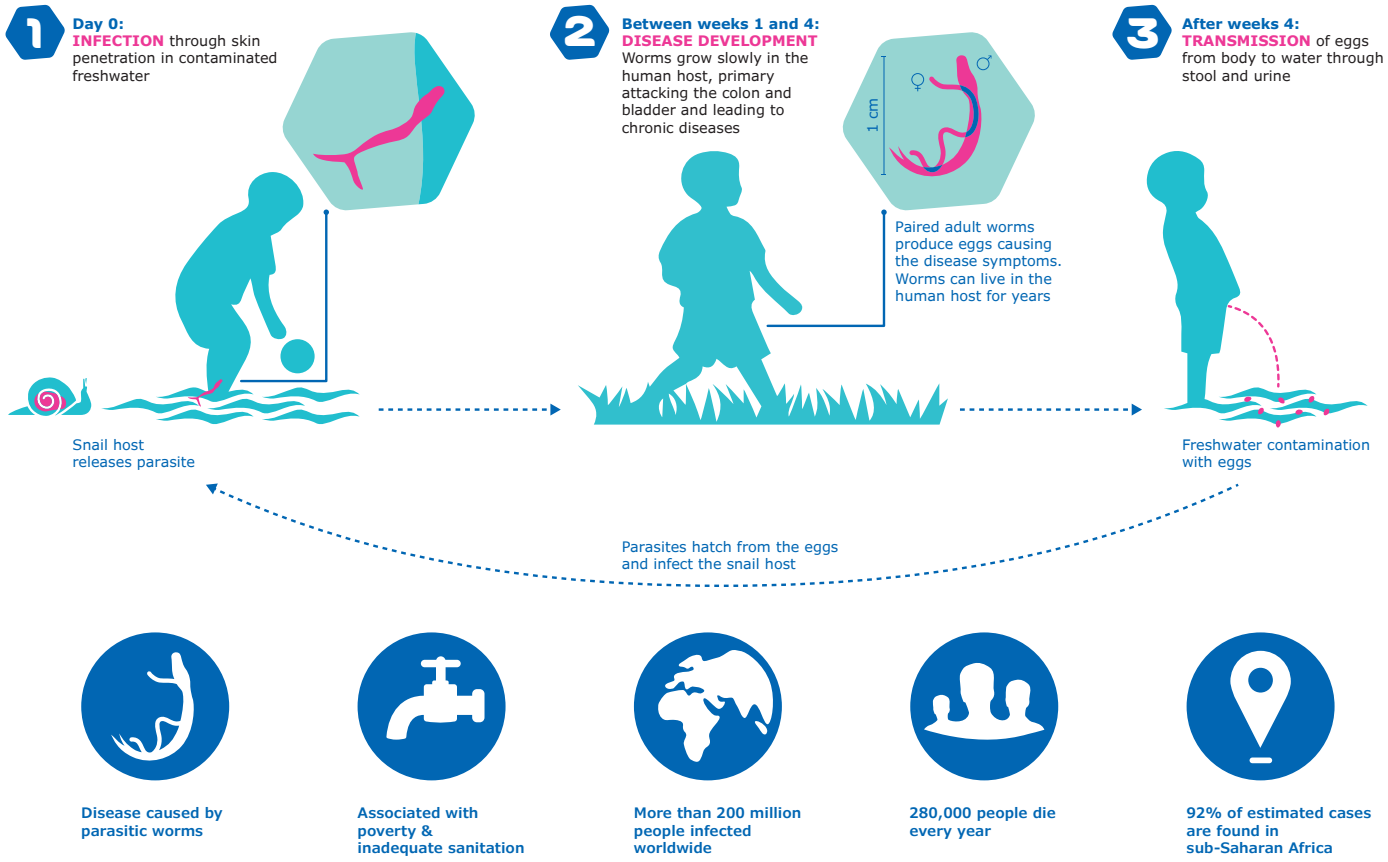
	Schistoma haematobium **(2/3 of global cases)**	**Schistosoma mansoni**
Pediatric cognitive and growth delays from anemia and inflammation	•	•
Hematuria, hydronephrosis, renal failure	•	
Bladder cancer	•	
Female genital Schistosomiasis	•	
Intestinal and liver fibrosis		•
Fulminant liver disease with hepatitis C co-infection		•

Today, the prevailing treatment option for persons with Schistosomiasis is the use of a medication called praziquantel (PZQ), which targets the parasitic worms, larvae and eggs. This acylated quinoline-pyrazine treatment therapy has a proven therapeutic and safety index. Treatment with PZQ has been the cornerstone of the WHO-promoted global strategy against Schistosomiasis for several decades.

While this therapy is highly effective toward reducing the worm burden in afflicted patients, it cannot alone eliminate Schistosomiasis for a variety of reasons[3]:

- PZQ may not be effective in all patients, and it does not kill developing larvae
- It does not prevent reinfection, as humans continue to be exposed to infected waterways
- The medicine is still not available for everyone; In fact, today only an estimated 20% of school-age children who need PZQ currently receive it
- Public health experts worry that continued reliance on PZQ raises the risk of drug-resistant parasites emerging over time

Figure 1:
Schistosomiasis Propagation and Consequences



The effort to develop a Schistosomiasis vaccine

The development of a viable, affordable vaccine against Schistosomiasis is a critical objective in ongoing efforts to break the cycle of chronic reinfection, and end organ damage, cancer and female genital Schistosomiasis, particularly in poor communities that do not have access to safe drinking water, adequate sanitation and a viable personal hygiene infrastructure. Healthcare experts anticipate that the best long-term strategy will involve routine vaccination coupled with periodic drug treatment using PZQ.

This White Paper discusses the Schistosomiasis vaccine candidate *Sm*-TSP-2, and efforts that are underway to improve and optimize its initial production process, to make it viable and sustainable at a commercial scale. The *Sm*-TSP-2 vaccine candidate was developed by a consortium of partners and team of scientists spearheaded by the product development partnership (PDP) named Texas Children's Hospital Center for Vaccine Development (TCH-CVD), in the National School of Tropical Medicine at Baylor College of Medicine, and it has shown promising results in early-phase clinical trials[4].

The *Sm*-TSP-2 vaccine antigen is a 9-kilodalton (kDa) recombinant protein that corresponds to a surface protein from the worm *Schistosoma mansoni* (*S. mansoni*), which is one of six species of trematode worm of the genus Schistosoma. *S. mansoni* is to blame for the intestinal/liver form of Schistosomiasis, and is one of the two species that is responsible for approximately one-third of all Schistosomiasis cases, including all of the cases that occur in the Americas[5].

Through the successful application of RNA interference (RNAi) and other disruptive technologies, the PDP teams were able to ascribe special functions to the *Sm*-TSP-2 molecule and improve its role in reducing parasite survival[6, 7, 8]. The molecule has been scaled up for production and further testing.

Preclinical studies and early-phase (Phase Ib) clinical trial testing to assess safety, reactogenicity, immunogenicity and ascending dose of the candidate vaccine, with or without aduvant (AP 10-701) have shown that vaccination using this recombinant protein sub-unit can induce antibody and cellular immune response against intestinal Schistosomiasis and substantially reduce the worm burden in healthy exposed adults, in an endemic area of Brazil[9–12].

Improving the *Sm*-TSP-2 vaccine-production platform

The initial production process developed by the PDP TCH-CVD team was suitable to produce the small quantities needed for early-phase laboratory testing. However, to make the process amenable for larger-scale production, TCH-CVD has partnered with Merck KGaA, Darmstadt, Germany.

Through this collaborative partnership, a team of bioprocessing-oriented scientists, engineers and economists from Merck has been working in close technical collaboration with TCH-CVD to advance the following technical and business objectives:

- Conduct a thorough technical and economic review of the overall process and a critical evaluation of each step, to identify technical and economic bottlenecks
- Investigate, implement and validate several state-of-the-art technology alternatives to improve the performance of key unit operations
- Streamline the overall system and reduce the number of unit operations, using principles of process intensification and improved engineering integration
- Develop a robust, streamlineMid production paradigm that maximizes production yield of the vaccine antigen at commercial scale
- Reduce capital and operating costs (including materials and labor), with a goal of minimizing the per-unit cost of the final vaccine (using the benchmark cost of $1/dose as a target)
- Develop a detailed lifecycle cost analysis for the final production paradigm, to both quantify efficiency gains and cost savings resulting from specific technology upgrades, and establish a realistic per-dose cost basis for the final vaccine product
- Carry out robust economic analysis and modeling to identify not only the direct cost savings of the system upgrades, but predict potential longer-term socioeconomic and health-related savings that would result from mass immunization using this candidate Schistosomiasis vaccine

Cost containment is especially critical when it comes to neglected tropical diseases like Schistosomiasis, as mass-vaccination campaigns often aim to administer millions of doses through programs that are typically paid for by resource-constrained local governments, non-governmental organizations (NGOs), and public/private partnerships. For more discussion on the economic impact of the technology advancements discussed here, see the Box, Understanding the Vaccine's Full Economic Impact, on page 5.

Sm-TSP-2 initial vaccine-production process

TCH-CVD initial pre-clinical production route required the following steps:

- Fermentation in a yeast-based bioreactor
- Dilution of the viscous fermentation broth (which has 30% suspended solids content), using buffering agents to dilute and wash the stream in successive steps, in order to minimize fouling the hollow-fiber-membrane filtration and resin-bead chromatography steps that follow
- Clarification, purification and volume reduction of the stream via a two-step filtration process
- Separation, concentration and capture of the vaccine antigen from the clarified stream using several chromatography steps
- Elution of the packed chromatography columns to release and harvest the antigen product that was captured by the chromatography resin beads
- Formulate the antigen into a final injectable vaccine formulation

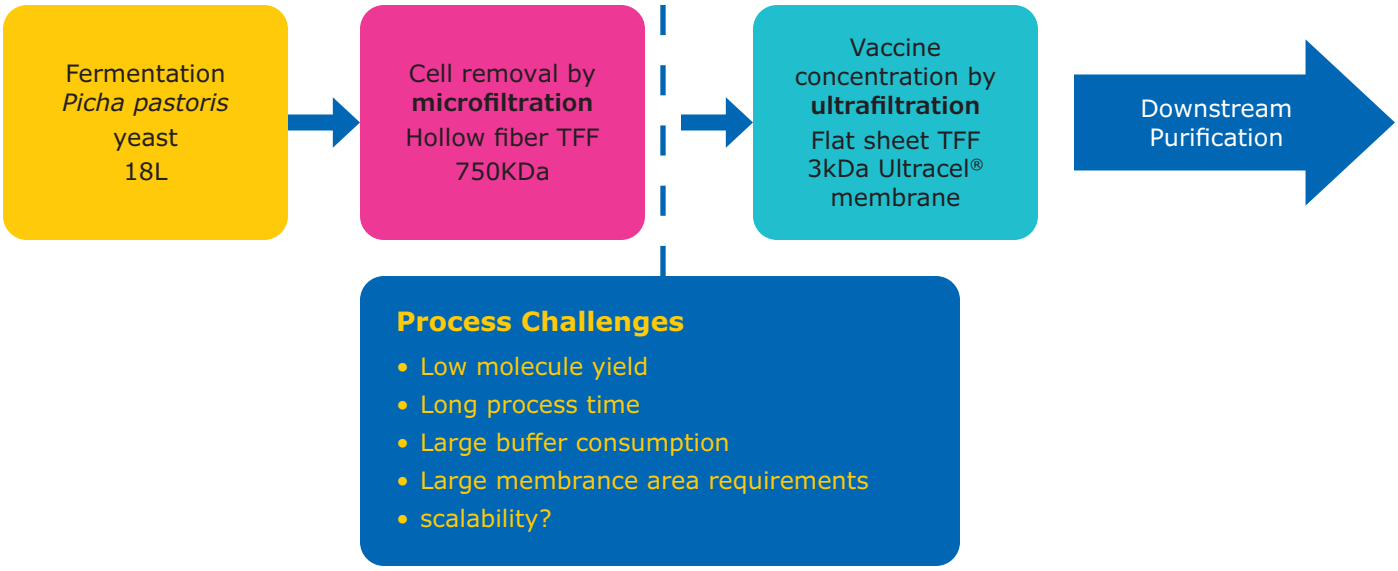
In the original process (Figure 2), the yeast-based bioreactor yields 18 liters per batch, with a suspended-solids content of 30%. To dilute and wash this stream ahead of the downstream filtration process, 12 liters of buffer must be added to each batch, to enable clarification using the two-step filtration process (to condition the stream ahead of the downstream chromatography process):

- *Step 1. Microfiltration using a hollow-fiber-membrane module.* This step removes unwanted cells by microfiltration, and is used to concentrate the expanded volume (after the 17-liter batch is expanded by adding 12 liters of added buffering agent) back down to the target volume of 12 liters
- *Step 2. Ultrafiltration using a flat-sheet, tangential flow filtration (TFF) module.* This step is used to further concentrate the batch down to the final target volume of 7.2 liters

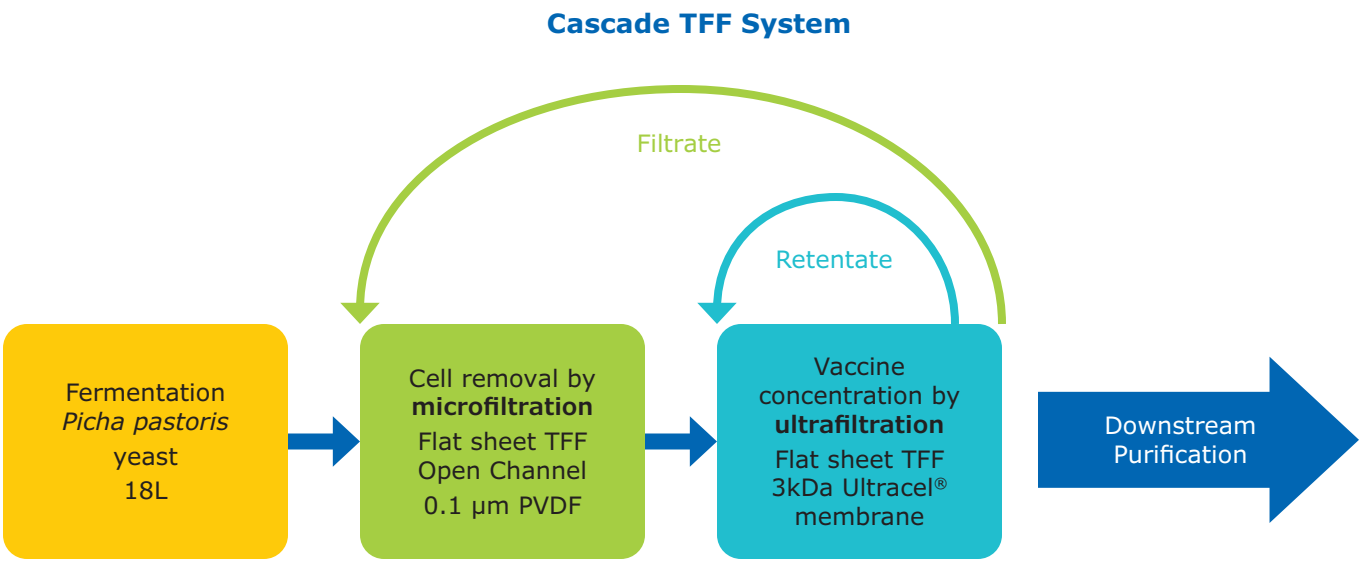
This multi-step clarification process, involving successive cycles of dilution and washing, proved to be inherently inefficient, resulting in relatively large losses of the target vaccine antigen at each step. The initial production process produced a purified stream of *Sm*-TSP-2 that had a yield of just 31% target antigen at 97% purification.

Figure 2:

Previous Clarification Process



Revised Clarification Process



Process Improvements

- Reduce complexity due to combining previsouly separate TFF steps
- Fouling at MF step is reduced due to continuous dilution of feed by permeate buffer recycled from the UF step
- Reduce buffer use
- Smaller membrane area requirements
- 1.7X highter concentration factor achieved
- Shorter Process Time (Shorter Suite Time)
- Smaller Manufacturing Footprint

Understanding the vaccine's full economic profile

Neglected tropical diseases often do not get the attention they need from large, multinational vaccine developers, because such products rarely provide a strong business case or sufficient profit motive to satisfy corporate objectives or shareholder expectations with regard to return-on-investment (ROI). Instead, vaccines and therapeutic interventions aimed at neglected tropical disease often rely on altruistic, humanitarian drivers, and are thus more likely to be championed by NGOs and public-private partnerships, such as the one described here. As such, the need to minimize overall production costs through engineering advances and optimization efforts is of paramount importance.

During such efforts, ongoing economic analysis and modeling should strive to quantify not only the cost savings that can result from manufacturing advances, but to the potential large-scale economic impact that can result from the successful treatment of chronic, debilitating and life-threatening conditions. Such savings come in the form of offset healthcare costs, reduced morbidity and mortality, and increased economic productivity for the community at large. Such a robust economic assessment provides a strong "artificial business case" that can help to entice private-public investment and government commitment to address neglected topical diseases.

Experts in MilliporeSigma's Value Management Center (VMC) work closely with drug- and vaccine developers, applying principles of Lean Methodology and Value Stream Mapping to evaluate processes holistically, and develop a business case that quantifies:

- The net cost reduction and financial impact on per-dose vaccine cost associated with the proposed technology improvements and process-optimization efforts
- The potential economic impact of shorter production timelines, higher production yields, lower capital and operating costs, improved speed to clinic, simplified regulatory compliance and so forth
- The potential socio-economic impact of the proposed upgrades to an established process in terms of the socioeconomic impact and offset healthcare costs that arise due to expanded access to the vaccine or lifesaving therapy
- Guidance and best practices to inform the selection of the most appropriate technologies for future applications

When MilliporeSigma's VCM team analyzed the process upgrades being pursued for *Sm*-TSP-2 Schistosomiasis vaccine, yielded demonstrable cost savings. Going forward, VCM analysis will confirm the anticipated economic impact of the chromatography-related advances that are currently being explored.

As shown in Figure 3, the process revisions discussed here yielded direct economic impact—specifically by eliminating the need to add buffering agents to dilute the bioreactor supernatant through the use of a state-of-the-art cascade TFF filtration and clarification process (A commercial-scale version of the initial process would easily have required thousands of liters of buffering agent per year). The team used the following assumptions:

- Six bioreactor runs per year
- The Pellicon and Prostak filter cartridges used in the new cascade TFF filtration process can each be used for 30 production runs before they need to be replaced

The upgraded filtration and clarification system alone (and the elimination of the dilution step needed in the original process), yielded the following cost reductions:

- *Cost of chemicals and consumables* — reduced by 98% compared to the initial process
- *Cost of labor* — reduced by 38%
- *Overall annual production cost* — reduced from $102,668 to $65,351, yielding a net savings of $37,317 per year
- *Process cost savings* — reduced by 36%
- *Amount of buffering agent required* — reduced by 88%
- *Labor hours* — down by 38%
- *Increased product recovery* — The revised product is able to recover 93% of the Sm-TSP-2 produced in the bioreactor (up from just 31% using the initial process)
- *Increased total yield* — These improvements allow the process to produce an additional 34 g of final drug product per batch run at commercial scale — representing a 36% increase in yield compared to the original process

Figure 3:
Increasing Access to Vaccine
Economic Impact of Process Revisions

Baseline Modelling

- Runs per Year = **6**
- Filter Reuses (Pellicon® cassette & Prostak® module) = **30**
- Filter Reuses (Hollow Fiber) = **1**
- Labor Hours per Run (Old) = **8**, (New) = **5**
- Buffer per Run (Old) = **92L**, (New) = **11L**
- Buffer Cost = **$0.50/L**
- Volume per Run = **20L**
- Titer = **1g/L**
- Product Recovery (Old) = **64%**, (New) = **93%**

Output

Cost Comparison ($/year) - HARD						
Unit Operation	**Old Process**		**New Process**		**% Difference**	
Chemicals and Consumables	$	-35,468	$	-851	-98%	
Labor	$	-7,200	$	-4,500	-38%	
Grand Total	$	-102,668	$	-65,351	-36%	
Estimated Hard Savings ($/year)			$	37,317		

Business Impact

- 36% Process Cost Savings $$$
- 88% of Buffer Saved $
- 38% Labor Hours Saved $
- **Potential for an Additional 34g of Final Drug Product (36% Increase) $$$$$**

*** VALUE IMPACT = $

Limitations of the initial process

To make the production process technically feasible and economically viable for commercial-scale production, the TCH-CVD/Merck team needed to implement technology and design-based upgrades to overcome several key limitations of the initial process:

- *Low molecule yield.* The initial, multi-step process was only able to achieve a protein-recovery yield of 31%
- *Long processing times, involving multiple unit operations.* This makes the process more complicated and less amenable to localized production in developing nations.
- *Large consumption of buffer fluids for dilution and washing.* The need to dilute the bioreactor supernatant creates inefficiencies, as it calls for an expanded fluid volume, longer processing times, consumption of costly buffering fluids, and reduced overall yield (as product losses occur at each of the successive dilution and volume-reduction steps)
- *Large membrane area requirements to ensure sufficient clarification of the fermentation broth with its relatively high viscosity and suspended solids content.* This adds capital costs and operating complexity to the system
- *Scalability issues.* Certain technology choices in the initial system (such as hollow-fiber-membrane filtration) do not scale up reliably

An analysis of the mass balance and purity of the *Sm*-TSP-2 produced using the initial multi-step process analysis[9] confirmed that major yield losses occur during two key steps — downstream clarification step of the fermentation broth from the bioreactor, and the multi-step chromatography process used for antigen capture. Using the amount of *Sm*-TSP-2 in the supernatant from the bioreactor as the basis (100% of the starting material), the yield fell to 79% as the percentage of total after the first filtration step, and 64% as the percentage of total after the second filtration step, and just 33% as the percentage of total after elution from the chromatography process.

Improvements focus on three areas

To advance a commercial-scale cGMP-compliant manufacturing process, the TCH-CVD/Merck team has focused its initial efforts on improving several key processing steps and reducing the total number of unit operations that are responsible for the largest productivity penalties and yield losses:

- The clarification process that is used to clarify the fermentation broth (which has a high suspended-solids content of 30%), and reduce its volume
- The chromatography steps that are used to separate and harvest the vaccine antigen from the clarified fermentation broth
- The additives needed for the final formulation were revised, to make them more suitable for large-scale production.

Each of the three major upgrades the team is making to the initial process is discussed in greater detail here.

Upgrade 1. Using cascade tangential flow filtration (TFF) for clarification and volume reduction.

To overcome the limitations of the hollow-fiber membrane filtration system used in the original process, the team evaluated the use of a cascade tangential flow filtration (TFF) system (Figure 4). Such a system carries out two TFF steps in a single unit operation. During operation, the permeate from the second TFF step is recycled continuously to the inlet of the first TFF step, to help wash and dilute the incoming, high-solids-content fermentation broth enough to enable filtration without high consumption of buffer.

The cascade TFF system used in the upgraded process combines MilliporeSigma's open-channel, membrane-based Prostak microfiltration modules with MilliporeSigma's 3kD Ultracel® ultrafiltration modules, and these modules are operated simultaneously. The resulting design provides an inherently compact, streamlined approach, which allows the upgraded process to efficiently concentrate each 18-liter supernatant batch to 8 liters, and eventually to 4.3 liters. This allows a cleaner, more-concentrated stream to be delivered to the downstream chromatography step — without the need to first expand the stream by first adding (and later removing) 12 liters of buffer fluid.

The cascade TFF system not only improves the clarification efficiency and reduces the total volume of buffer fluid needed, but it reduces capital and operating costs and processing time, reducing the clarification and filtration time from two shifts to a single shift. This upgrade also enables a smaller downstream chromatography system to be used (thanks to enhanced volume reduction during the filtration step).

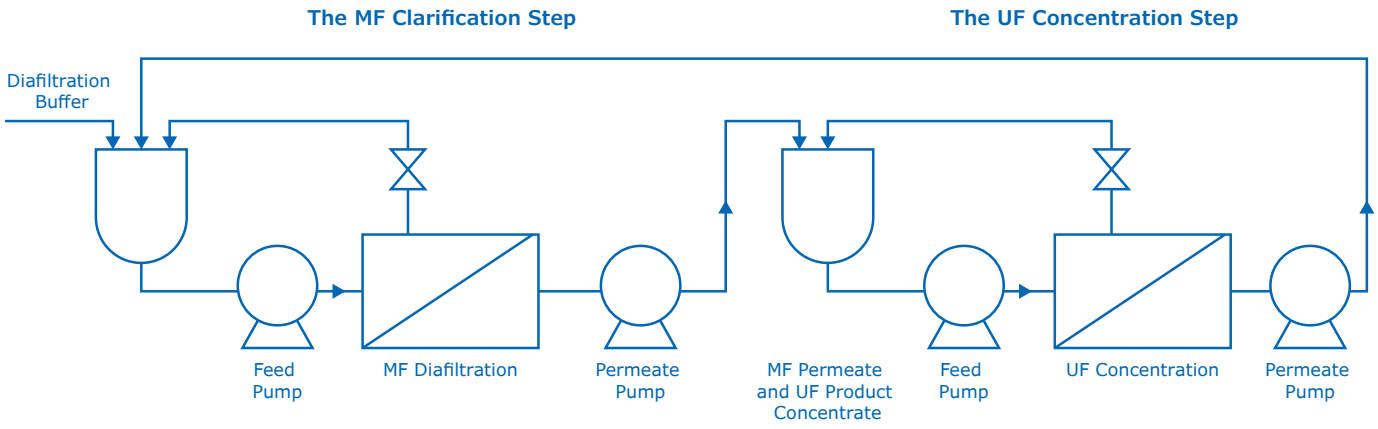
Upgrade 2. Improving the chromatography step

In the original process, the *Sm*-TSP-2 antigen binds to a cation exchange resin (based on an older, agarose-based chromatography resin), during a traditional bind-and-elute chromatography step. Majority of host cell proteins (Host cell proteins and other impurities) are collected in the column flow-through. After washing the column with Succinic acid buffer at pH 6 (~15 mS/cm), the Sm-TSP-2 molecules bound by the chromatography resin is eluted with a linear gradient using 500 mM sodium chloride.

While suitable to produce small-scale quantities of the antigen needed for the early, small-scale testing, the chromatography step in the original process provides relatively poor recovery yield (on the order of 50%). And, to cope with insufficient impurity removal, a second chromatography step is required for polishing.

Figure 4:
***Sm*-TSP-2 Production Cascade TFF system**

A Typical Cascade TFF System



Rather than two separate TFF operations, combine micro - and ultrafiltration into **single, linked process**

The Merck team is working to apply its extensive product, engineering, design and troubleshooting expertise to evaluate several competing options for improving chromatography step used in the initial system design. The initial process uses a two-step, resin-bead-based chromatography system. Ongoing investigation is under way to explore and test a variety of options, with a goal of both streamlining and maximizing product capture and reducing the two-step chromatography option to a single step.

Importantly, the redesign of the clarification process is already delivering a smaller, cleaner stream to the downstream chromatography process. Using today's state-of-the-art resins and column designs, the goal is to reduce the number of columns, and to maximize the number of bind-and-elute cycles before the resin must be replaced. The team expects that the final design upgrades in these two areas will help to improve the product-capture yield of the chromatography step (which is roughly 50% in the initial process), and further reduce the capital and operating costs of the commercial-scale manufacturing process *Sm*-TSP-2, enabling a more sustainable and appropriate design for developing nations aiming for localized Schistosomiasis vaccine production.

Upgrade 3. Focus on the vaccine formulation

The initial vaccine formulation produced by the TCH-CVD team used 10-milliimolar (mM) of the buffering agent imidazole, 15% sucrose and 2 of mM phosphate at pH 7.4. While this formulation was suitable for preclinical and early-phase testing, imidazole is not suitable for use in parenteral formulations, and is not readily available in GMP grade — potentially hindering the regulatory approval of the final vaccine formulation.

After investigating suitable alternatives, the Merck experts recommended replacing imidazole with histidine as the buffering agent of choice, to stabilize the proteins through mild buffering capacity and minimize unwanted protein-protein interactions that can lead to aggregation. The improved formulation is now comprised of 20 mM of histidine, 15% sucrose and 4 mM of phosphate at pH 7.4.

Histidine is a similar molecule to imidazole, with compatible biochemical aspects to imidazole (in terms of stability, pH, and other product characteristics), and functions similarly in terms of stabilizing the antigen. However, unlike imidazole, histidine has already been used in other vaccines that have transitioned successfully to the clinic (having received FDA approval with regard to safety and efficacy), and it is readily available in both parenteral formulations and GMP grade. These attributes should help *Sm*-TSP-2 Schistosomiasis vaccine to avoid potential regulatory hurdles.

Closing thoughts

While many factors will contribute to technical and economic viability of any commercial-scale vaccine-production process, the ability to optimize key parameters as early as possible in the process-development phase can have the largest long-term impact in terms of locking in operating flexibility, reducing overall costs, improving the likelihood of regulatory approval and minimizing the per-dose vaccine cost. The time to explore and validate relevant process improvements is when the prototype production process is still at small scale, as it always more difficult and more costly to revise the process later. Such performance objectives are essential for any biopharmaceutical process but become even more critical to enable the localized production and widespread administration of vaccines and lifesaving therapies aimed at reducing neglected tropical diseases in developing nations.

As demonstrated here, the ability to use state-of-the-art technology substitutions and advanced design concepts has helped the TCH-CVD team to improve clarification and volume reduction, reduce fouling in filtration and chromatography steps, reduce the volume of buffer fluids and the overall membrane required, reduce and simply the final system design, and improve overall antigen-recovery rates, concentration factor and purity thresholds, paving the way for a commercial-scale, cGMP-compliant production paradigm for this candidate Schistosomiasis vaccine.

The long-term technical collaboration between the TCH-CVD and Merck experts continues to provide demonstrable advantages for both partners, allowing all stakeholders to bridge scientific and engineering knowledge gaps (and essentially establishing a *a de facto* scientific advisory board) that exploit synergies to overcome critical hurdles that can hamper process development and scaleup, product formulation, critical economic assessment and ongoing training, leveraging that collaborative effort to bring promising, affordable vaccines and lifesaving therapies to market more quickly.

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