ImmunoVida: A Recombinant Quadrivalent Influenza Vaccine for Latin America

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On our honor as University Students, we have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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1. Executive Summary

ImmunoVida is a recombinant quadrivalent influenza vaccine designed to provide broad protection against influenza A and B subtypes in individuals aged 18 and older. This facility, located in São Paulo, Brazil, aims to produce 90 million doses per year. The upstream process includes an Sf9 insect cell seed train infected with purchased baculovirus across eight 10,000 L bioreactors, yielding 1.56 kg of HA antigen per run. These harvested cells proceed to a downstream purification train that begins with two centrifugation steps, followed by anion exchange chromatography, cation exchange chromatography, ultrafiltration, diafiltration, hydrophobic interaction chromatography, viral filtration, and a final diafiltration step. The final drug product is filled into single-dose glass vials. The proposed plant will produce nine batches per year, yielding 18.08 kg of HA (4.52 kg of each strain) annually. It will operate for 18 weeks of the year, with the remainder of the year dedicated to research and development activities, seed train expansion, and rented to outside companies for other applications of the baculovirus expression system.

Economic analysis confirms strong financial viability of the facility. The total capital investment is estimated at $101.7 million, with annual operating costs of $129.3 million per year. Depending on market conditions, yearly revenue is projected between $315 million to $705 million. A 10-year financial production across best, mid, and worst-case scenarios shows return of investment (ROI) ranging from 1026% to 3773%, confirming the profitability of the project and viability to investors.

2. Introduction

Influenza remains a significant global health challenge with 3-5 million severe cases and up to 650,000 deaths annually (World Health Organization, 2023). Seasonal influenza epidemics disrupt productivity and overwhelm healthcare systems, particularly in developing regions like Latin America where healthcare infrastructure is more limited. The most effective strategy to reduce the spread of influenza and reduce hospitalizations remains to be the widespread distribution of vaccines (Center for Disease Control and Prevention, n.d.).

There are four main types of influenza viruses: A, B, C, and D. Influenza A and B are the strains that cause the seasonal flu in humans, Influenza C infects humans but does not cause major outbreaks, and Influenza D is not known to affect humans (Center for Disease Control and Prevention, 2024). The A strain has historically been the only influenza virus known to cause pandemics, one of which was the 1918 Spanish Influenza Pandemic. This event was responsible for 45-50 million deaths, prompting the creation of the first influenza vaccine (Gentile et al., 2019). Because of the pandemics and seasonal epidemics, strains are continuously monitored by the World Health Organization (WHO).

The major ways this virus can spread is by coming into direct contact with respiratory droplets, bodily fluids, or contaminated surfaces. People are most contagious one to three days after symptoms develop. Some of these symptoms can include: fever, cough, headache, fatigue, sore throat, stuffy nose, or body aches. Anyone, including healthy people, can get the flu but groups at a higher risk of developing serious complications include: adults 65 years and older, people with chronic conditions, pregnant individuals, those that have a body mass index greater than or equal to 40, and children younger than five years old (Centers for Disease Control and Prevention, n.d.). This is why the best prevention against influenza virus is mass vaccination.

2.1. Previous Technology

Traditional influenza vaccine production cultivates the live virus within embryonated chickens' eggs. The virus is then inactivated chemically, purified, and processed into a vaccine. While this process has been well-established for over 70 years, there are many limitations such as potential spread of the live virus, harm to people with egg-allergies, and labor-intensive manufacturing that is difficult to scale up within a six to eight month production cycle. Due to the long production cycle, a new strain circulating cannot have the fastest response readiness (Institute of Medicine (US) Forum on Medical and Public Health Preparedness for Catastrophic Events, 2010).

One emerging method has been recombinant technology, which uses insect cells infected with baculovirus to produce hemagglutinin (HA) proteins. Flublok was the first approved influenza vaccine to use this technology, producing a well tolerated quadrivalent vaccine containing four times more HA than other vaccine production methods (Cox et al., 2008). The baculovirus-insect cell expression system has become widely used in recombinant protein production over the past 20 years and addresses several limitations of other manufacturing routes including elimination of a live virus allowing for increased safety and specificity of the HA protein, easier growth conditions achieving higher protein yields, highly scalable, and rapid response to novel circulating strains. Given the advantages demonstrated in Flublok's FDA-approved production method, a similar approach has been taken for production of ImmunoVida.

2.2. Product: ImmunoVida

ImmunoVida is a quadrivalent recombinant protein vaccine design similar to that of Flublok, aimed at increasing accessibility of this technology to Latin America. Each dose will feature four HA antigens, two influenza A subtypes and two influenza B subtypes, recommended by the WHO and global influenza programs based on the most prevalent strains circulating. This vaccine is produced from Sf9 cells which are derived from the fall armyworm *Spodoptera frugiperda*. The HA proteins are recognized as foreign by the immune system, and specific hemagglutination inhibition antibodies are produced as a humoral immune response. This provides initial protection against the virus by allowing the body to more quickly and effectively defend against disease (Padilla-Quirarte et al., 2019). Studies showed that the high-dose recombinant vaccine resulted in 15% fewer cases in older patients than the common, egg-based vaccine (Kaiser Permanente Division of Research, 2023).

2.3. Project Scale

To meet regional demand and increase vaccine accessibility, ImmunoVida will be manufactured in São Paulo, Brazil to leverage existing infrastructure and the expertise of Intituto Butantan, the largest vaccine producer in Latin America. The proposed facility will match Instituto Butantan's 2023 production capacity of 90 million doses annually.

3. Discussion of Proposed Manufacturing

3.1. Process Flow Diagram

The manufacturing process of ImmunoVida involves several unit operations and pieces of auxiliary equipment. The process flow diagrams shown in Figure 3.1-1 and Figure 3.1-2 display both the equipment and stream layouts for this process. Production begins with upstream fermentation, consisting of shake flasks and bioreactors, followed by downstream purification, which includes centrifugation units, chromatography columns, and filtration units. Finally, the product is formulated and aseptically filled into glass vials on a filling line. A comprehensive list of equipment tags and their descriptions is provided in Table 3.2-1.

Figure 3.1-1. Upstream Process Flow Diagram

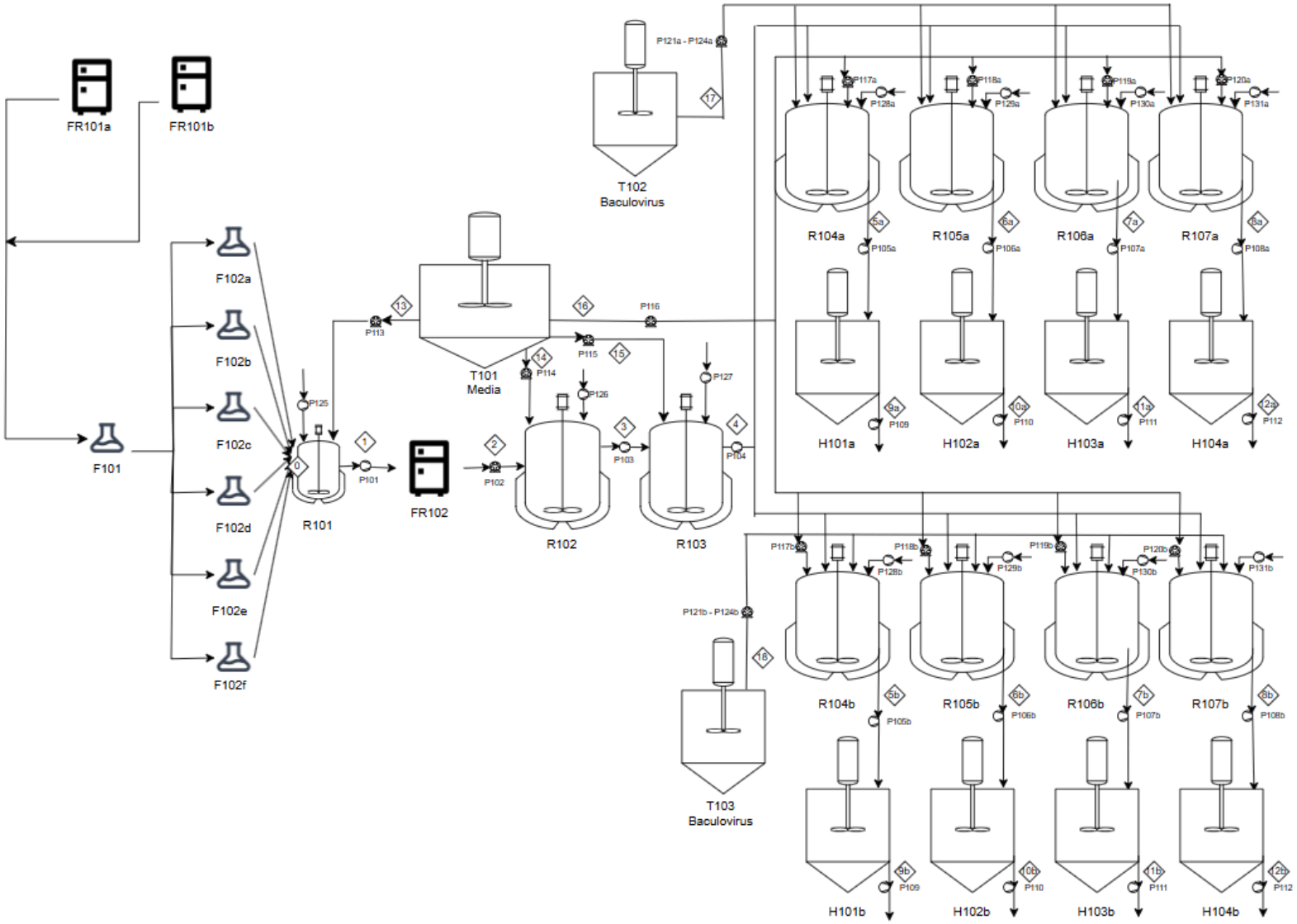
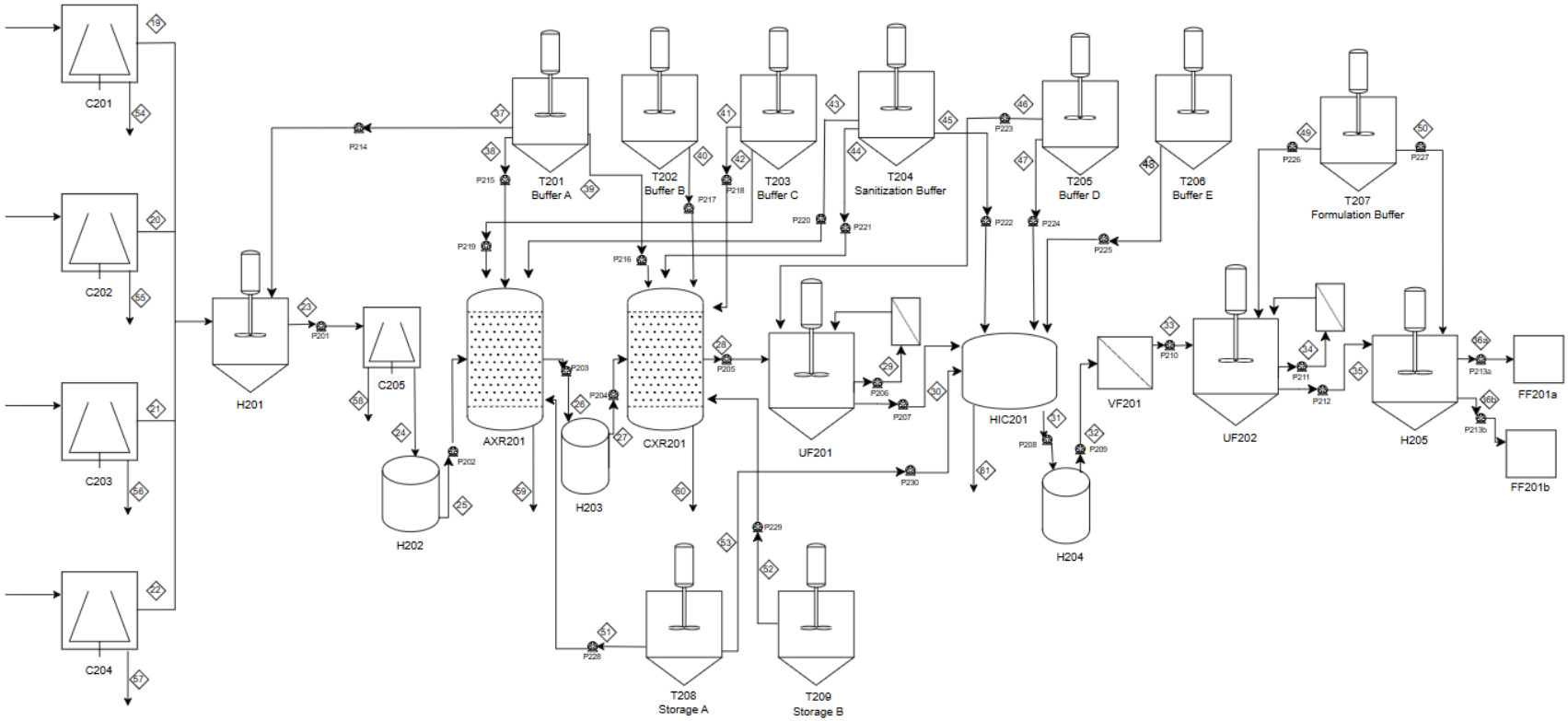


Figure 3.1-2. Downstream Process Flow Diagram



3.2. Overall Material Balance

The goal of our project is to manufacture 90 million doses of ImmunoVida annually, aligning with the 2023 output of Instituto Butantan, the leading influenza vaccine manufacturer in Latin America (Instituto Butantan, n.d.). Each dose consists of two influenza type A antigens and two influenza type B antigens, each weighing 0.0496 mg, for a total antigen content of 0.198 mg/dose. To meet our production goal, 17.82 kg of total antigen must be produced annually. This can be achieved with nine batches over a 18-week production season which means each batch must produce 1.98 kg of antigen. To create the material balances, we worked backwards from this target, incorporating the percent yields of each piece of equipment involved in the manufacturing process. A comprehensive overview of all the equipment yields is provided in Table 3.2-1.

After the HA antigen is produced through fermentation in the upstream process, it is purified using the downstream equipment. The cell slurry entering the downstream process contains the antigen, spent media, host-cell DNA, and other cellular debris from the fermenters. This slurry is fed into an initial round of centrifugation consisting of four centrifuges (C201-C204) to extract the HA antigen from the cell broth. This centrifugation step has a 95% yield and removes the spent media as waste. The resulting cell “pellet” is then resuspended in an extraction buffer containing 20 mM sodium phosphate, 1.0 mM EDTA, 0.01 % Tergitol-NP9, and 5% glycerol with a pH of 5.89. The mixture is homogenized in mixing tank H201, which, like all mixing and holding tanks, is assumed to have a 100% yield. The final concentration of the mixture is 28.4 mg solids/mL, resulting in a highly viscous solution composed of hydrated cell debris and the target antigen. This mixture is then transferred into another round of centrifugation (C205) for clarification, where the antigen is separated from most of the host-cell

DNA and other cellular debris. This step also achieves a 95% yield. The clarified solution is then pumped into holding tank H203 where it is stored before the next step.

Since the antigen is already suspended in the first extraction buffer, it is directly sent to the first chromatography column, an anion exchange column operated in flowthrough mode (AXR201). The eluate from this column, containing the antigen in the first extraction buffer, is temporarily stored in holding tank H203 before being sent to CXR201, a cation exchange column operated in bind-and-elute mode. The antigen is eluted from this column using a second extraction buffer containing 20 mM sodium phosphate, 0.03% Tergitol, and 5% glycerol with a pH of 7.02. Both of these chromatography steps have a 99% yield and remove any remaining host-cell proteins and genetic material from the antigen.

The antigen solution, now suspended in the second extraction buffer, is transferred from CXR201 to the ultrafiltration and diafiltration system UF201. In the ultrafiltration step, the solution is concentrated approximately 4.6-fold, followed by diafiltration, which exchanges the second extraction buffer with a third extraction buffer containing 20 mM sodium phosphate and 0.5 M ammonium sulfate at a pH of 7.0. Both steps have a yield of 97%, resulting in an overall system yield of 94.1%. Next, the solution is pumped into a hydrophobic interaction chromatography column (HIC201), operated in bind-and-elute mode to remove any antigen aggregates that may have formed. The antigen is eluted from this column using a fourth extraction buffer containing 20 mM sodium phosphate and no ammonium sulfate at a pH of 7.0. This chromatography step achieves a 92% yield. After being briefly stored in holding tank H204, the solution then undergoes a viral filtration step with a 99% yield. Following this, another diafiltration system (UF202) replaces the fourth extraction buffer with the final formulation

buffer, which primarily contains WFI along with excipients. This step has a 97% yield, and the final formulation composition is detailed in Table 3.5.1-1.

Finally, the finished drug substance goes through an aseptic filling line (FF201a - FF201b) and is transferred into stoppered glass vials, resulting in the final drug product. The filling and packaging line is assumed to have an 85% yield, with 10% of the losses attributed to material loss during startup and stoppage, as well as residual product in the pipes, and a 5% loss due to packaging defects. In addition to these process losses, an intentional 10% overfill is applied to each vial to ensure that, after stopper placement and potential evaporative or volumetric loss, each unit still meets the labeled dosage volume. This overfill volume is not considered a loss and is accounted for throughout the material balance to ensure sufficient drug substance is available to meet final fill requirements.

Table 3.2-1. Hemagglutinin Antigen Overall Yield

Equipment Tag	Equipment Description	Antigen Yield (kg/batch)	Antigen Yield (kg/year)	Step Yield	Cumulative Yield
F101	5 L Shake Flask	N/A	N/A	100.00%	100.00%
F102a - F102f	5 L Shake Flasks	N/A	N/A	100.00%	100.00%
R101	200 L Fermenter	N/A	N/A	100.00%	100.00%
FR101 - FR102	Freezers	N/A	N/A	100.00%	100.00%
R102	2000 L Fermenter	N/A	N/A	100.00%	100.00%
R103	12000 L Fermenter	N/A	N/A	100.00%	100.00%
R104a - R107a R104b - R107b	10000 L Fermenters	3.12	28.08	100.00%	100.00%
P101 - P230	Pumps	3.12	28.08	100.00%	100.00%
H101a - H104a H101b - H104b H201	Drug Substance Mixing Tanks	3.12	28.08	100.00%	100.00%
T101 - T209	Media, Baculovirus, and Buffer Mixing Tanks	N/A	N/A	100.00%	100.00%
H202 - H204	Non-Mixing Holding Tanks	3.12	28.08	100.00%	100.00%
C201 - C204	Centrifuges	2.96	26.68	95.00%	95.00%
C205	Centrifuge	2.82	25.34	95.00%	90.25%
AXR201	Anion Exchange Chromatography	2.82	25.34	99.99%	90.24%
CXR201	Cation Exchange Chromatography	2.82	25.34	99.99%	90.23%
UF201	Ultrafiltration & Diafiltration	2.65	23.84	94.09%	87.52%
HIC201	Hydrophobic Interaction Chromatography	2.44	21.93	92.00%	84.90%
VF201	Viral Filtration	2.44	21.93	99.99%	78.11%
UF202	Diafiltration	2.36	21.27	97.00%	78.10%
H205	Final Formulation Tank	2.36	21.27	100%	75.76%
FF201a - FF201b	Filling & Packaging Line	2.01	18.08	85%	64.39%

3.3. Upstream

3.3.1. Cell Line and Baculovirus Expression System Selection

The production of this recombinant quadrivalent influenza vaccine utilizes *Spodoptera frugiperda* (Sf9) cells, which are insect cells derived from the ovaries of the fall armyworm. These cells serve as the host for the baculovirus expression vector system (BEVS), a well-established platform for the production of recombinant proteins. Recombinant baculoviruses are rod-shaped enveloped dsDNA viruses with genomes ranging between 80 and 180 kilobases. In this system, the recombinant baculoviruses infect host cells and hijack the cells' machinery to produce the desired recombinant protein (Marintcheva, 2017). The hemagglutinin antigen (HA antigen) is the protein of interest in this BEVS application, as it is a key surface protein found on the outer membrane of influenza viruses and induces a strong immune response in vaccine recipients.

The selection of Sf9 cells was driven by their ability to grow easily and be scaled up in serum-free suspension cultures, with well-documented ability in supporting high-yield recombinant protein production for complex viral proteins such as hemagglutinin (Hitchman et al., 2011). Additionally, production using Sf9 cells offers greater safety compared to other techniques such as traditional egg-based inactivated influenza production, as the recombinant baculoviruses used are non-pathogenic to humans.

BEVS also offers rapid and efficient production timelines, as only the baculovirus genome must be modified annually to match circulating influenza strains, rather than altering the cell line genome. As a result, large cell populations can be amassed in the off season in preparation for production. This lends the proposed facility unique flexibility and minimizes turnaround time between the selection of flu strains and antigen production. Although the

baculovirus stock is able to be produced in-house using Sf9 cells, the extensive additional purification processes required to isolate the baculoviruses fall outside the scope of this project. The cultivation of the baculovirus will be outsourced to a contract development and manufacturing organization (CDMO), with a designated budget allocation to cover this expense.

3.3.2. Seed Train Expansion

The flexibility of BEVS allows for the accumulation of large quantities of Sf9 cells in the pre-flu season. These cells can then be expanded through a seed train process, ensuring genetic stability of the working cell line and cutting down on fermentation time during the production season. The establishment of a working cell bank from the master cell bank is outside the scope of this project and is assumed to take place outside of the production season. The process-scale seed train expansion begins with the thawing of cells from the well-characterized cryopreserved Sf9 working cell bank at minimum of approximately 3×10^5 cells/mL (ThermoFisher Scientific, 2020). The cells are grown in ideal conditions until they approach a maximum density of 4×10^6 cells/mL (ThermoFisher Scientific, 2020).

The required antigen output per fermentation run was retrofitted by taking into account the length of the flu season and the expected yield of the downstream purification train, discussed at length in Section 3.2. To meet production goals for a 18-week season, nine batches were required, with each batch consisting of two fermentation runs. Each run produces 0.78 kg of flu type A antigens and 0.78 kg of flu type B antigens. Antigen yield per cell is dependent upon the conditions under which the cell culture is infected with the baculovirus, and was estimated based on the process established by Buckland et al. (2014). A yield of 20 mg of HA antigen per L of fermentation broth was assumed under the following conditions: the cell density

at infection was 2.0 x 10^6 cells/mL, the multiplicity of infection (MOI) was 1, and the fermentation continued for 50 hours post infection (HPI).

An MOI of 1 was selected based on previous work cited by Buckland et al. (2014), in which a scalable, high-yielding fed-batch process was developed to produce recombinant hemagglutinin using a similar *expres*SF® cell line (Meghrous et al., 2009). The productivity and viability of the insect cell line post-infection are closely linked to the MOI. An excessively high MOI can cause excessive cell stress, accelerate nutrient depletion during the post-infection stage, and lead to premature cell lysis (Alizadeh et al., 2024). Conversely, a low MOI may result in inefficient infection of the culture.

With the end conditions and yield determined, the calculated required volume of cell broth per fermentation run was roughly 80,000 L: 40,000 L for one strain of flu type A and another 40,000 L for one strain of flu type B. To counteract potential mass transfer limitations in oxygen transfer, eight 10,000 liter tanks were selected for the final infection stage. Prior to baculovirus infection, cells for both strains will be grown together in two parallel trains. The cell density requirements above and a doubling time of 27 hours were used to back-calculate the number and the volume of the fermentation stages required to achieve the desired yield. The doubling time was approximated from the range of 24-30 hours provided by ThermoFisher Scientific (2020). The proposed stages are tabulated below in Table 3.3.2.-1.

Table 3.3.2.-1. Seed Train Specifications

Equipment Tag	Working Volume (L)	Cell Seeding Density (cell/mL)	Maximum Cell Density (cells/mL)	Incubation Time (hours)
F101	5	2.44×10^5	1.95×10^6	81
F102a - F102f	5	3.26×10^5	2.60×10^6	81
R101	200	3.91×10^5	3.13×10^6	81
R102	2000	3.13×10^5	2.50×10^6	81
R103	12000	4.17×10^5	3.33×10^6	81
R104a - R107a R104b - R107b	10000	5.00×10^5	2.00×10^6	54

3.3.3. Bioreactor Specifications

Initial cell expansion occurs in a 5 L working volume master shake flask (F101), with its contents later distributed across six additional 5 L shake flasks (F102a - F102f). Six separate shake flasks were chosen instead of a single 30 L bioreactor due to their simplicity and the convenience of batch operation at this smaller scale. Bioreactor specifications for the rest of the fermentation train were chosen based on a similar industrial scale process for the FluBlok vaccine (Buckland, 2014). For each reactor, the working volume is 80% of the total volume with a height to diameter aspect ratio of roughly 1.1 to 1.3 and impeller diameters of roughly 0.35 to 0.5 times tank diameter (Buckland, 2014). Reactor geometries are displayed in Table 3.3.3-1.

Special considerations were taken to minimize damage to cells by minimizing shear stress. In accordance with Buckland, it was assumed that impeller tip speeds could not exceed 1.58 m/s (2014). To further minimize shear, bioreactors were fitted with low shear marine impellers. To mitigate the low impeller speeds, bioreactors were also fitted with four baffles to

promote homogenized mixing. Operating conditions and mixing considerations are further discussed in Section 3.3.5.

Table 3.3.3.-1. Bioreactor Geometries

Equipment Tag	Tank Volume (L)	Working Volume (L)	Height (m)	Tank Diameter (m)	Impeller Diameter (m)	Number of Impellers	Wetted Height (m)
R101	250	200	0.701	0.620	0.310	1	0.662
R102	2500	2000	1.651	1.280	0.457	2	1.554
R103	15000	12000	2.945	2.337	0.889	2	2.798
R104a - R107a R104b - R107b	12500	10000	2.772	2.200	0.837	2	2.631

3.3.4. Fed-Batch Fermentation Model

In the pharmaceutical industry, various reactor types are available and are selected based on factors such as growth kinetics, cell sensitivity, and cell lysis. This process specifically utilizes fed-batch bioreactors due to several key advantages. First, continuous nutrient feeding promotes sustained cell growth and metabolism without overwhelming cells with excessive nutrient concentrations at once. Fed-batch bioreactors also enable the effective management of oxygen levels and pH in high-density cultures. Additionally, the accumulation of harmful byproducts is minimized compared to traditional batch cultures. This is especially important for our process, as the recombinant Sf9 cells lyse after infection with the baculovirus.

Equation 3.3.4-1 for fed-batch systems was used to calculate both the initial seeding media requirement and the flow rate for subsequent media addition throughout the fermentation process in each reactor. The growth of Sf9 cells is primarily influenced by the availability of substrates that fuel cell metabolism. Although multiple substrates are necessary for cell growth, the accumulation of biomass is constrained by the availability of the limiting substrate. This

system uses Hink's TNM-FH Insect Medium sourced from Sigma Aldrich, where the limiting substrate, lactalbumin hydrolysate (LAH), is present at a concentration of S_0 = 3.33 g/L. $Y_{X/S}$, the yield of biomass per gram of substrate, was determined to be 1.5 x 10^9 cells/g based on literature values from a similar Sf9 system (Käßer et al., 2022). The starting cell count and the final cell count at the end of each reactor's growth cycle, X_0 and X_T, respectively, are tabulated in Table 3.3.2-1. based on the aforementioned cell density requirements for seeding, passaging, and infection. The growth time, t, for each reactor is 81 hours, except for the infection reactors, where cell growth was assumed to be negligible after infection, resulting in a growth time of 54 hours.

The media flow rate, F, was computed for each reactor, and these calculations are summarized Table 3.3.4-1. The total amount of media fed to each reactor was found by multiplying the media feed rate by the growth time. The seeding media volume was calculated by subtracting both the cell seed stock volume (the final working volume from the previous reactor step) and the amount of media fed from the final working volume of each reactor.

$$X_T = X_0 + FY_{X/S}{}^* S_o t \qquad \textbf{Equation 3.3.4-1}$$

A notable exception was made for the infection bioreactors, where the final media volume in the tanks was set to be 100 L less than their stated working volume. This adjustment was made to provide sufficient headspace for the addition of the baculovirus stock, which will be sourced from a CDMO as a high-titer stock with a concentration of 1 x 10^8 PFU/mL (Invitrogen life technologies, 2002). Based on an MOI of 1 and the aforementioned baculovirus stock concentration, it was calculated that 97 L of stock would be needed for each infection reactor. However, this amount was rounded up to 100 L for simplicity and to ensure that the working volume of each reactor reaches 10,000 L prior to being sent for purification.

Table 3.3.4.-1 Fed-Batch Specifications

Equipment Tag	Final Working Volume (L)	Seed Stock Volume (L)	Initial Amount of Media Added (L)	Media Flow Rate (L/hr)	Total Amount of Media Fed (L)
R101	200	30	61	1.35	110
R102	2000	200	924	10.81	876
R103	12000	2000	2993	86.51	7007
R104a - R107a R104b - R107b	10000	1500	5397	55.61	3003

3.3.5. Agitation Specifications and Oxygen Requirements

To replenish the oxygen consumed during cell growth, air must be continuously sparged into each reactor. The amount of air required is dependent on the oxygen uptake rate (OUR) of the Sf9 cells and the efficiency of oxygen transfer from the gas phase to the liquid (media) phase, which is described by the volumetric mass transfer coefficient, kLa. The minimum kLa threshold for each reactor was calculated using the theoretical kLa equation, Equation 3.3.5-1a, and these values are shown in Table 3.3.5-1. OUR was calculated by multiplying X, the maximum cell count per reactor (the final cell count) by the specific oxygen uptake rate of Sf9 cells, q_{O_2}. This value was approximated to be 0.198 mmol O_2 per g biomass per hour using the average of four measurements of Sf9 oxygen consumption and an assumption that the dry weight of one Sf9 cell is 0.926 nanograms (Pamboukian et al., 2008; Palomares & Ramirez, 1996; Wagner et al., 2011). The saturated dissolved oxygen concentration at 27 ^{o}C, C^{*}, was determined to be 7.96 mg/L (Hamilton, 2025). The target dissolved oxygen concentration, C_L, was set to 4.776 mg/L, or 60% of the saturated value according to the scale up process validated by Buckland et al. (2014).

$$OUR = X * q_{O_2} = k_L a(C^* - C_L)$$ **Equation 3.3.5-1a**

Following the calculation of target kLa, operating conditions for each reactor were carefully selected to meet oxygenation requirements while avoiding damaging the cells with excessive shear. As shown in Equation 3.3.5-1b, the kLa values of the individual bioreactors are dependent upon P_g, the gassed power input, and v_s, the superficial velocity of the gas moving through the reactor. These were manipulated by regulating impeller speed and air flow rate to the reactor.

$$kLa = 0.026 * (\frac{P_g}{V})^{0.4} * v_s^{0.5}$$ **Equation 3.3.5-1b**

Gassed power input, P_g, is dependent upon impeller speed (N) and power requirement (P) of the impeller without aeration. The ratio between P_g and and P was assumed to be 0.925 in accordance with the values reported in the bioreactors designed by Buckland et al. and their constant impeller flow number of 1.4 (2014). Power requirements were calculated using Equation 3.3.5-2, then multiplied by 0.925 to find P_g. The density of the cell broth at the highest cell density is approximately 1006 kilograms per cubic meter. The power number was fixed at 3, as exemplified by Buckland et al. (2014). The diameter of the tank impeller is represented by D_i. For bioreactors with multiple impellers, the power requirement was multiplied by the number of impellers.

$$P = N_p \rho N^3 D_i^5$$ **Equation 3.3.5-2**

In order to avoid prematurely lysing the Sf9s due to excessive shear, all impeller speeds were set such that the tip of any given impeller would not exceed a speed of 1.58 m/s. This value was selected because it was the maximum impeller tip speed used by Buckland et. al (2014). Tip speed was calculated using equation 3.3.5-3 below.

$$u_{tip} = \frac{\pi DN}{60}$$ **Equation 3.3.5-3**

Superficial velocity is defined as the volume of gas being fed to the bioreactor divided by the cross-sectional area of the tank. Once an ideal impeller speed was selected, air feed rate was toggled to adjust superficial velocity to reach the target kLa. The final operating conditions for each bioreactor are tabulated below in Table 3.3.5.-1.

Table 3.3.5.-1. Bioreactor Mixing and Aeration Operating Conditions

Equipment Tag	Working Volume (L)	Impeller Speed (rpm)	Tip Speed (m/s)	Target kLa (hr^{-1})	Actual kLa (hr^{-1})	Air Feed (vvm)	Reynolds Number
R101	200	97	1.57	5.8	29.9	0.2015	5197
R102	2000	44	1.05	4.6	22.8	0.0294	5136
R103	12000	36	1.58	5.2	76.2	0.0295	14095
R104a - R107a R104b - R107b	10000	34	1.58	6.2	80.4	0.0279	15018

Notably, the kLa targets are far exceeded by the actual kLas in every bioreactor. This is due to two primary reasons: carbon dioxide management and mixing limitations due to shear. As discussed previously, dissolved carbon dioxide, a byproduct of cellular metabolic activities, must remain at a level below 100 mmHg. This is mitigated by sparging in excess air, lowering the relative presence of carbon dioxide in the bioreactor and increasing CO_2 stripping. This action also helps to explain Buckland et al.'s constant impeller flow number of 1.4, where flow number is defined in Equation 3.3.5-4 below. In this case, Q is defined as the volumetric flow rate of air to the system, N is the impeller speed in rev/s, and D_i is the impeller diameter in m. By holding impeller flow number constant, Buckland et al. (2014) set a direct relationship between impeller operating conditions and impeller diameter (and tank geometry by extension) to a designated

oxygen flow rate, which was mimicked in the proposed design as a way to counteract CO_2 accumulation in the cell culture.

$$N_a = \frac{Q}{ND_i^3} \qquad \textbf{Equation 3.3.5-4}$$

Secondly, the shear sensitivity of the cells places limits on mixing capabilities. It is evident that in R101 and R102, the Reynolds numbers are not high enough to bely a well mixed bioreactor in a turbulent regime. The additional sparged air will cause turbulence itself, supplementing the mixing done by the impeller. Both of these goals will be achieved using microspargers lining the walls of the bioreactor, providing very fine bubbles that are effective at improving CO_2 stripping and promoting low-shear mixing (ThermoFisher Scientific, 2020).

3.3.6. Upstream Duration and Yield

From end-to-end, a single fermentation run is expected to take roughly 520 hours. For the purpose of this report, a single fermentation run is defined as the time taken to expand cells from the first 5 L shake flask through completion of infection with the baculovirus in the eight 10,000 L bioreactors. The final product will consist of 40,000L of cell broth for one flu type A antigen and 40,000 L of cell broth for one flu type B antigen. The timeline of one such run is shown in Table 3.3.6-1 below.

Table 3.3.6-1. Production Time Table for Single Fermentation Run

	Equipment Tag	Working Volume (L)	Time in Reactor (hr)	Transfer Time (hr)	Elapsed Time (hr)
Off-Season Expansion	F101	5	81	0.25	82
	F102a - F102f	5	81	0.25	164
	R101	200	81	0.25	247
Flu-Season Production	R102	2000	81	1	331
	R103	12000	81	8	415
	R104a - R107a	10000 (Type A)	104	4 (total)	519
	R104b - R107b	10000 (Type B)			

The expected final products from *half* of a fermentation run are shown below in Table 3.3.6-2. Each fermentation run produces 40,000 L of broth per antigen strain, and two antigen strains are produced per run. It was elected to present fermentation products this way, as purification is run in 40,000 L batches and all strains must be kept separate until formulation. To present products as one 80,000 L batch would create unnecessary confusion and imply that the products of Infection Complex A were intermixable with the products of Infection Complex B at this stage.

Table 3.3.6-2. Characterization of Products of Half of a Fermentation Run

Substance	Quantity
Antigen	0.78 kg
Cell Matter	1211 kg
Fermentation Broth	40000 L

3.4. Downstream

3.4.1. Centrifugation

There are two centrifugation steps at the beginning of the purification: extraction and clarification. Extraction is the process of recovering the product of interest, the HA antigen, from the cell broth. Following a resuspension after extraction, clarification occurs. This is the process of removing impurities and isolating the product in liquid. Centrifugation was used to achieve these tasks due to the sheer volume of each batch and concerns about cell debris clogging depth filtration filters.

While various types of centrifuges are used in the pharmaceutical industry, disc stack centrifuges were selected for this process due to their ability to provide continuous and efficient separation, enabled by their high surface area for separation. These centrifuges operate by rapidly rotating a series of closely spaced conical disks, generating strong centrifugal forces that effectively separate lighter and heavier components within the feed mixture. During one purification run, this requires the resuspension of 0.74 kilograms of antigen in 8475 liters of buffer. For each centrifugation step, antigen yield was assumed to be 95%.

Both the extraction and clarification step can be modeled in Equation 3.4.1-1, where v_g is the sedimentation velocity, g is gravity, Q is the volumetric processing rate, n is the number of plates, ω is the rotational speed, Θ is the angle of the disks, R_o is the outer disc radius, and R_i is the inner disc radius.

$$Q = \frac{v_g 2\pi(n-1)\omega}{3g} cot\theta(R_o^3 - R_i^3) \qquad \textbf{Equation 3.4.1-1}$$

The extraction process separates the cell media from the desired HA antigen, DNA, and other cell debris. This requires a Westfalia Type Disc Stack Centrifuge Separator that has 110 discs all angled at 45 degrees, an outer disc radius of 0.977 m, and an inner disc radius of 0.275

m. In order to ensure recovery of relevant solids, the settling velocity of the antigen (the lightest species of interest) was used to determine centrifuge operating settings. The settling velocity of the HA antigen was assumed to be 8.00 x 10^{-6} centimeters per hour, in agreement with proteins of similar size. The fastest processing time of one of the above Westfalia centrifuges for 40,000 L of antigen amounted to over 100 hours, so the extraction step was scaled out to four of the described centrifuges, each processing one of the four 10,000 L bioreactors producing the strain being purified. A description of the extraction centrifuges is tabulated below in Table 3.4.1-1.

Table 3.4.1-1. Extraction Centrifugation (Per Centrifuge)

	Unit	Value
Equipment Tag	-	C201 - C204
Number of Discs (N)	-	110
Disc Angle (θ)	°	45
Outer Radius (R_o)	m	0.977
Inner Radius (R_i)	m	0.275
Centrifuge Speed	rpm	4,484
Volumetric Processing Rate	L/hr	374.25
Volume Fed	L	10,000
Processing Time	hr	26.72
Cell Debris Recovery	%	98
Antigen Recovery	%	95

Following the first round of centrifugation, the discarded media is sent to waste, while the remaining material is resuspended in an extraction buffer . The buffer consists of 20 mM sodium phosphate, 1.0 mM EDTA, 0.01 % Tergitol-NP9, and 5% glycerol and has a pH of 5.89, as

described in Wang et al. (2006). This buffer is added to the cell pellet, resuspending it to a concentration of 28.4 milligrams of solids per milliliter for the extraction step (Wang 2006). During one purification run, this requires the resuspension of approximately 241 kilograms of antigen and cell debris in 8475 liters of buffer. Antigen yield was assumed to be 95% in both centrifugation steps.

Following extraction and resuspension, the antigen and cell debris mixture is clarified to remove cell solids prior to entering chromatography. In this case, everything but the antigen must be concentrated into a cell pellet. The supernatant containing the extraction buffer and antigen are collected and sent to anion chromatography.

The average settling velocity of the cell debris was unknown and instead approximated using the centrifugation setting of Wang et al. (2006) and assumptions made about the lab-scale centrifuge they used. The settling velocity was approximated as 0.01 centimeters per hour. Given the much smaller volume for this step, one single centrifuge was deemed capable of handling the liquid volume. However, rotational speed was intentionally lower than what was possible in order to slightly increase processing time to relieve the burden on the workers starting up and shutting down the machine. The final specifications of the centrifuge used for clarification are tabulated below in Table 3.4.1-2.

Table 3.4.1-2. Clarification Centrifugation C205

	Unit	Value
Number of Discs (N)	-	40
Disc Angle (θ)	°	45
Outer Radius (R_o)	m	0.44
Inner Radius (R_i)	m	0.15
Centrifuge Speed	rpm	3,280
Volumetric Processing Rate	L/hr	4291
Volume Fed	L	8,475
Processing Time	hr	2.03
Antigen Recovery	%	95

While considerations were made for cleaning, there is adequate time included in the production schedule to do so (Section 4.5). Thus, cleaning time was excluded from total processing time, as it does not impede the progression of the batch through the purification train. Following this stage, the pellet containing the cell debris is safely disposed of. The supernatant and HA antigen are kept in a holding tank before continuing to anion exchange chromatography. Approximately 0.7 kilograms of antigen in 8500 liters of buffer leave this step.

3.4.2. Anion Exchange Chromatography

The next step in the downstream process is anion exchange chromatography, which separates components based on their net charge. Anion exchange chromatography acts as a "scavenger" in this part of the process, capturing negatively charged genetic material and native cell proteins and allowing the desired antigen to flow through (Wang et al., 2006). This process

assumes an antigen yield of 99.99% (Buckland et al., 2014), with 0.70 kg of antigen in roughly 8500 L of Buffer A leaving this step. The volume lost in this stage is considered to be negligible.

The chromatography steps were based on a lab-scale purification process of the HA antigen (Wang et al., 2006) and an industrial-scale production process of the Flublok vaccine (Buckland et al., 2014). The 300 L anion exchange column (AXR201) (Table 3.4.2-1), will be packed with Cytiva Capto Q ImpRes resin.

The operating velocity of this chromatography stage was chosen to be 60% of the maximum operating velocity of 700 cm/hr, as recommended in the Cytiva manual for the Capto Q ImpRes resin (Cytiva Life Sciences, 2024). This was chosen in order to account for possible pressure build up in the column due to accumulation of debris in the resin. The manual also requires that operating pressure drop not exceed 3 bar in the column. To verify this, the Kozeny-Carman equation for pressure drop through a packed bed was used to calculate the estimated pressure drop given the other operating conditions (Equation 3.4.2-1).

$$\Delta P = \frac{150\,(1-\varepsilon_e)^2}{d_p^2 \varepsilon_e^3} \times \eta \times L \times u \qquad \textbf{Equation 3.4.2-1}$$

In order to compute pressure drop, the external porosity, ε_e was assumed to be 0.35. Pore diameter, d_p, was 90 micrometers per Cytiva. The viscosity of the mixture was assumed to be slightly higher than water, given the high amounts of genetic material within, at 1.1 x 10^{-3} Pascal-seconds. The pressure drop was found to be 0.47 bar, which is well within range. This value and other column specifications are tabulated in Table 3.4.2-1 below.

Table 3.4.2-1 Anion Exchange Column AXR201 Specifications

Parameter	Value
Volume (L)	300
Height (m)	0.2
Diameter (m)	1.38
Pore Diameter (μm)	90
Linear Velocity (cm/hr)	420
Operating Temperature (°C)	20
Operating Pressure Drop (bar)	0.47
Yield (%)	99.99

The standard operating schedule for AXR201 is included below in Table 3.4.2-2. Importantly, no buffer exchange is required prior to chromatography because the binding buffer is identical to the extraction buffer used in the centrifugation stages. Buffers are named for simplicity, but their specific compositions can be found in Table 11-1. Four cycles are required to process all 8500 L of product.

Table 3.4.2-2. Anion Exchange Chromatography AXR201 Operating Schedule

Step Name	Buffer	Volume (CV)	Time (min)
Equilibrate	Buffer A	5	14.3
Load	Buffer A	7.2	20.6
Clean	Buffer C	4	11.2
Sanitize	Sanitization	5	14.3
Regenerate	WFI	10	14.3

As suggested by Wang et al., there is no elution step for anion exchange chromatography (2006). This is attributed to there being no desirable products on the column. Instead, the column is cleaned and regenerated using Buffer C and Sanitization buffers, followed by WFI. Approximately 0.7 kilograms of antigen in 8500 liters of buffer exit this column.

3.4.3. Cation Exchange Chromatography

Anion exchange chromatography is followed by cation exchange chromatography, which also separates based on net charge. The antigen binds to the resin, and unwanted residual cell debris passes through. The 300 L cation exchange column (CXR201) (Table 3.4.3-1) is packed with negatively charged Cytiva Capto SP ImpRes resin. Pressure drop for this column was also calculated using Equation 3.4.2-1 with the same assumptions made about linear velocity, external porosity, and viscosity.

Table 3.4.3-1 Cation Exchange Column CXR201 Specifications

Parameter	Value
Volume (L)	300
Height (m)	0.2
Diameter (m)	1.38
Pore Diameter (μm)	90
Linear Velocity (cm/hr)	420
Operating Temperature (°C)	20
Operating Pressure Drop (bar)	0.47
Yield (%)	99.99

The standard operating schedule for CXR201 is included below in Table 3.4.3-2. The product exits anion exchange chromatography already suspended in the binding buffer for cation exchange chromatography, thus no buffer exchange is required. Four cycles are required to process all 8500 L of product.

Table 3.4.3-2. Cation Exchange Chromatography CXR201 Operating Schedule

Step Name	Buffer	Volume (CV)	Time (min)
Equilibrate	Buffer A	5	14.3
Load	Buffer A	7.2	20.6
Wash	Buffer A	7	20.0
Elute	Buffer B	6	17.1
Clean	Buffer C	4	11.4
Sanitize	Sanitization	5	14.3
Regenerate	WFI	5	14.3

The antigen yield for this process is assumed to be 99.99%, with 0.70 kg of antigen exiting the column in 7200 L of Buffer B (Table 11-1).

3.4.4. Ultrafiltration and Diafiltration

An ultrafiltration and diafiltration (UF/DF) step (UF201) is then required to concentrate the cation exchange outlet stream and to conduct buffer exchange from Buffer B to Buffer D, ensuring buffer compatibility for the subsequent hydrophobic interaction chromatography step (Section 3.4.5). Another diafiltration step (UF202) is also required between viral filtration (Section 3.4.6) and formulation and filling (Section 3.5) to exchange Buffer E to the final formulation buffer. Hollow fiber tangential flow filter cartridges with a filter area of 6 square

meters from Cytiva are used for these processes. These filters have a 10,000 nominal molecular weight cutoff (NMWC), which is small enough to effectively exclude the HA antigen (63 kD), ensuring it remains in the retentate while allowing the buffers to pass through the filters.

To set θ_p, the protein rejection coefficient - a critical filter specification for all UF/DF systems - Equation 3.4.4-1 and Equation 3.4.4-2 for batch diafiltration were first solved as a system of equations for UF202.

$$\left(\frac{C}{C_o}\right)_{salt} = exp\left[\frac{-V_D}{V}(1 - \theta_B)\right] \qquad \textbf{Equation 3.4.4-1}$$

$$Yield = exp\left[\frac{-V_D}{V}(1 - \theta_p)\right] \qquad \textbf{Equation 3.4.4-2}$$

Assuming a θ_B of zero (the buffers completely pass through the filters), a yield of 97%, an 855 L input volume from viral filtration, and a 99% antigen purity, θ_p was calculated to be 0.9934 for the Cytiva filter. This calculation also yielded 3937 L of the final formulation buffer needed for this step. Additionally, Cytiva recommends the flowrate through the filter to not exceed 30 L per square meter per hour. A filter area of 18 m² which corresponds to three 6 m² Cytiva filters and a total processing time of 15 hrs satisfies this recommendation per Equation 3.4.4-3, where u_p represents permeate flux, A represents total filter area, and t represents time. The processing parameters for UF202 are tabulated in Table 3.4.4-2. Approximately 0.6 kg of antigen in 855 L of formulation buffer leaves this step per run. Since the output from UF202 is more concentrated than what is needed for final formulation, an additional 762.6 L of formulation buffer is added in-line between this step and the final formulation mixing tank (H205).

$$u_p = \frac{V_D}{At} \qquad \textbf{Equation 3.4.4-3}$$

The specifications for ultrafiltration and diafiltration step UF201 were back calculated using the aforementioned design equations. Equation 3.4.4-4 and Equation 3.4.4-5 for batch ultrafiltration, where CF represents the concentration factor, were also utilized. The established protein rejection coefficient of 0.9934 for UF202 was retained to calculate CF and subsequently specify V_F.

$$Yield = CF^{\theta_P - 1} \qquad \textbf{Equation 3.4.4-4}$$

$$CF = \frac{V_o}{V_F} \qquad \textbf{Equation 3.4.4-5}$$

As this UF201 includes both ultrafiltration and diafiltration, each with an assumed yield of 97%,, the combined yield of the unit is 94.09%. The processing parameters for UF201 are tabulated in Table 3.4.4-1. Approximately 0.7 kg of antigen in 1563 L of Buffer E leave this step per run.

Table 3.4.4-1. Ultrafiltration and Diafiltration UF201 Processing Parameters

Parameter	Ultrafiltration	Diafiltration
Buffer Rejection Coefficient	0	0
Protein Rejection Coefficient	0.9934	0.9934
Concentration Factor	4.605	-
Input Volume (L)	7200	1563
Output Volume (L)	1563	1563
Dilution Volume (L)	-	7200
Total Filter Area (m^2)	18	18
Permeate Flux (m/s)	6.173×10^{-6}	6.173×10^{-6}
Processing Time (hr)	14.09	18.00
Yield (%)	97	97

Table 3.4.4-2. Diafiltration UF202 Processing Parameters

Parameter	Diafiltration
Buffer Rejection Coefficient	0
Protein Rejection Coefficient	0.9934
Concentration Factor	-
Input Volume (L)	855
Output Volume (L)	855
Dilution Volume (L)	3937
Total Filter Area (m^2)	18
Permeate Flux (m/s)	4.051×10^{-6}
Processing Time (hr)	15.00
Yield (%)	97

3.4.5. Hydrophobic Interaction Chromatography

Hydrophobic interaction chromatography (HIC201) serves as a final polishing step to remove residual impurities, particularly any dimers or trimers that may have formed during the previous chromatography steps. The HA antigen is prone to aggregation when subjected to pH shifts or high local protein concentrations - both of which primarily occur during the cation exchange step. As the antigen binds to the resin at high concentrations and is subsequently eluted through a pH shift, these conditions can promote aggregate formation.

Unlike ion exchange chromatography (IEX) which separates based on net charge, HIC separates based on hydrophobicity, removing highly hydrophobic aggregates from the HA antigen monomer. Generally, HIC resins are selected based on the target ligand's hydrophobicity. Capto Butyl ImpRes resin from Cytiva was chosen for this process based on lab-scale experiments done by Li et al. (2018), in which they investigated the binding of the Hepatitis B antigen (HBc-VLP) on a variety of resin types including butyl, octyl, and phenyl-based resins.

Since our HIC column operates in bind-and-elute mode, a butyl-based resin was selected based on its ability to bind over 99% of the target antigen under high-salt conditions, while binding less than 10% of the antigen and maintaining sufficient waste protein binding at zero-salt conditions (Li et al., 2018). The buffers in this step were also modeled from the experiments done by Li et al. (2018). The specifications of HIC201 are shown in Table 3.4.5-1.

The pressure drop calculation was performed in the same manner described in Section 3.4.2 and using Equation 3.4.2-1. However, given that this polishing step should contain almost exclusively buffer and the desired product, viscosity was assumed to be the same as water. Extraparticle porosity was assumed to be 0.35. The linear velocity was selected using 60% of the maximum operating velocity.

Table 3.4.5-1. HIC Column HIC201 Specifications

Parameter	Value
Volume (L)	57
Height (m)	0.2
Diameter (m)	0.602
Pore Diameter (μm)	40
Linear Velocity (cm/hr)	210
Operating Temperature (°C)	20
Operating Pressure Drop (bar)	0.21
Yield (%)	92

A 20 mg/mL resin loading capacity was assumed for this column (Cytiva Life Sciences, 2023), meaning that all 1563 L of solution containing 0.659 kg antigen entering the column per run can be loaded onto the column at once and processed in a single cycle. The operating

schedule is described in Table 3.4.5-2. The yield of this step is assumed to be 92%, with approximately 0.606 kg of antigen exiting the column in 855 L of Buffer E (Table 11-1).

Table 3.4.5-2. HIC Column HIC201 Operating Schedule

Step Name	Buffer	Volume (CV)	Time (min)
Equilibrate	Buffer D	10	57.1
Load	Buffer D	27.43	156.7
Wash	Buffer D	10	57.1
Elute	Buffer E	15	85.7
Sanitize	Sanitization	5	28.6
Regenerate	WFI	5	28.6

3.4.6. Viral Filtration

Viral filtration ensures the removal of any remaining viral contaminants. This process passes the antigen in a buffer through a 160 kDa molecular weight cut-off (MWCO) membrane filter designed to remove particles larger than the target protein (63 kDa). The system consists of one filter totaling a filter area of 6 square meters, with the liquid passing through at a volumetric flow rate of 129.6 L/hr. A recovery of 99% is assumed due to the significant difference between the antigen molecular weight and the MWCO of the membrane. Due to the direct flow nature of this filter, volume reduction is considered negligible. The Cytiva Ultipor™ VF grade DV20 virus removal filter has been selected for this stage. The exit stream will contain 0.60 kg of HA antigen in 855 L of Buffer E. The specifications for the viral filtration are tabulated below in Table 3.4.6-1.

Table 3.4.6-1. Viral Filtration VF201 Specifications

Parameter	Value
Antigen Output (kg):	0.60
Volume Processed	855
Total Filter Area (m^2):	6
Operating Pressure (bar)	2.10
Typical Flux (LMH)	21.60
Volumetric Flow Rate (L/hr)	129.60
Permeate Flux (m/s):	2.16×10^{-2}
Processing Time (hr):	6.60
Antigen Yield:	0.99

3.5. Formulation and Filling

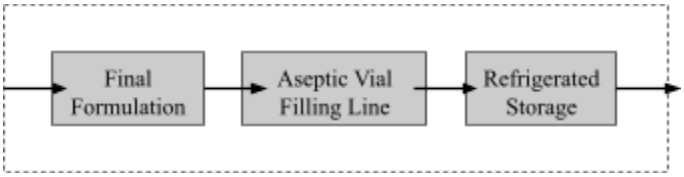


Figure 3.5-1. Formulation and Fill-Finish Block Flow Diagram

3.5.1. Final Formulation

Table 3.5.1-1. Formulation

Component	5500 L Batch (kg)	0.55 mL Vial (mg)
HA (4 strains) in WFI up to Volume	1.98 (0.495 each)	0.198
Sodium chloride	48.4	4.84
Sodium phosphate monobasic	2.2	0.22
Sodium phosphate dibasic	5.5	0.55
Tween 20	0.3025	0.03025

In addition to purified antigens, WFI, and residual trace materials from the manufacturing process, vaccines contain small amounts of excipients. Excipients serve specific functions: Maintain pH, prevent contamination, and extend shelf life. Our excipient selection is based on the Flublok formulation. Due to the high antigen dose in our quadrivalent vaccine (four times a standard flu shot), an adjuvant was not selected.

The vaccine ingredients are combined and uniformly mixed under controlled conditions in a dedicated mixing tank before being transferred to the aseptic vial filling line.

3.5.2. Aseptic Vial Filling Line

Aseptic conditions are critical for ensuring vaccine safety, quality, and efficacy. In the pharmaceutical industry, approximately 80% of recalls are linked to packaging-related issues (GlobeNewswire, 2024). The fill-finish process for ImmunoVida is fully automated, employing advanced equipment within a cleanroom environment under continuous skilled operator oversight. Automation minimizes human intervention, reducing the risk of contamination.

Key manufacturers in aseptic fill-finish technology include AST, Bausch+Strobel, Dara Pharma, and Groninger. The leading bulk vial filling systems can process up to 600 vials per minute (36,000 per hour). These machines wash, dry, and sterilize glass vials using an optimized washing process and a sterile tunnel. The filling is performed using SmartFill technology with 100% In-Process Control (IPC), ensuring precise dosing through pre- and post-fill weight measurements. Vials are then sealed with sterile stoppers and caps, with built-in fault detection systems ensuring quality. Industry experts estimate an 85% yield from the fill-finish process (Martagan et al., 2020).

Following sealing, vials are externally cleaned and loaded onto trays for transport to storage. The aseptic filling machine is designed for a cleanroom environment, incorporating

isolators and restricted access barrier systems (RABS) in compliance with current Good Manufacturing Practices (cGMP). These machines can be programmed for various products, and optional mixing and filtration can be integrated into the filling path. Selecting the correct-sized fill line is crucial to maximizing efficiency. High-speed lines (300–600 vials per minute) are available, with models processing 200–400 or 400–600 vials per minute. The faster equipment can reduce production time for a 10-million-dose batch by approximately two weeks (13.6 days vs. 40.9 days for slower equipment). To avoid bottlenecks, we propose utilizing two 600-vial-per-minute lines for this scale.

3.5.3 Vial Selection

We have selected Type I borosilicate glass vials, known for their chemical stability and suitability for pharmaceutical applications. Ready-to-sterilize vials, stoppers, and caps will be procured in bulk from suppliers. Single-dose vials were selected to ensure quality, however it results in a high portion of raw material costs.

3.5.4 Refrigerated Storage

Vaccines will be labeled with proper identification and usage information, as shown in Figure 3.5.4-1. Finished doses will be stored in a specialized vaccine refrigerator, maintaining temperatures of 2°C to 8°C (36°F to 46°F), before distribution to clinics.

Figure 3.5.4-1. Example Label

3.6. Ancillary Equipment

3.6.1. Pumps

Pumps are essential to the safe and aseptic transport of fluids through the facility. While centrifugal pumps are the traditional choice, special considerations need to be made when dealing with shear-sensitive materials such as cells and antigens. For this reason, the pumps in the proposed facility consist entirely of air-driven compressors and low-shear peristaltic pumps.

Air driven compressors were selected to sparge air into bioreactors during fermentation and to drain the large bioreactors, limiting shear on the delicate Sf9 cells while maintaining a large volumetric flow rate to clear the bioreactors for the next use.

Peristaltic pumps were selected to move the remaining process fluids, buffers, and cell media due to their low-shear capabilities and ease of cleaning. More specifically, no process process fluid comes in contact with the pump itself, only the tubing snaked through it. As a result, the tubing can easily be discarded and incinerated following use, rather than investing in costly safety precautions for CIP caustic. This concept is further discussed in sections 3.4.4 and 6. In addition, peristaltic pumps can be used to connect a variety of fluid start points and destinations with new tubing, meaning they are far more flexible than pumps with fixed tubing. Lastly, peristaltic pumps require no control valves and thus have a smaller pressure drop than more traditional pumps and thus cost less energy to operate.

The pump selections for this design are tabulated below, with the air-powered compressors tabulated in Table 3.6.1-1a and 3.6.1-1b, and peristaltic pumps in 3.6.1-2. A constant pressure loss of 0.5 atm to friction in tubing was assumed. One control valve was allotted to every non-peristaltic pump, each contributing 0.5 atmospheres of pressure drop to the system. Gravity pressure head was approximated using a conservative estimate of the distance

from the floor to the top of the destination equipment. Required outside air flow was calculated using Equation 3.6.1-1. Air is assumed to behave ideally.

$$P_1V_1 = P_2V_2 \qquad \textbf{Equation 3.6.1-1}$$

Annual operating costs were calculated using the average cost of electricity for businesses in Brazil in 2024, averaging 0.101 USD per kilowatt hour (Global Petrol Prices, 2025).

Table 3.6.1-1a Overview of Compressors for Bioreactors

Equipment Tag	Purpose	Flow Rate (L/hr)	Power (W)	Operating Time (hr/yr)	Energy Use (kWh/yr)	Cost of Operation (USD/yr)
P101	Draining	800	17.98	4.5	0.08	0.01
P103	Draining	2000	70.86	18.0	1.28	0.13
P104	Draining	12000	683.92	18.0	12.31	1.20
P105 - P108 a & b	Draining	20000	1141.53	9.0	10.27	1.04
P109 - P112	Draining	374	16.78	961.9	16.14	1.63
P125	Sparging	2418	138.16	1458.0	201.43	20.34
P126	Sparging	3528	203.11	1458.0	296.14	29.91
P127	Sparging	20066	1145.35	1458.0	1669.92	168.66
P128 - P131 a & b	Sparging	17732	1010.84	1872.0	1892.29	191.12

The operating requirements for compressor-powered tank draining are included below in Table 3.6.1-1b. Required outside air flow was calculated using equation 3.6.1-1. Air is assumed to behave ideally.

$$P_1V_1 = P_2V_2 \qquad \textbf{Equation 3.6.1-1}$$

Table 3.6.1-1b Overview of Operating Requirements for Compressors

Equipment Tag	Outlet Flow Rate (L/hr)	Required Air Pressure (atm)	Inlet Flow Rate (L/hr)
P101	800	1.50	1202
P103	2000	1.79	3586
P104	12000	2.28	27308
P105 - P108 a & b	20000	2.28	45551
P109 - P112	374	2.00	750
P125	2418	2.28	5510
P126	3528	2.29	8074
P127	20066	2.28	45703
P128 - P131 a & b	17732	2.28	40358

Peristaltic pumps were selected for every process except for bioreactor draining, as their variable volumetric flow rates permitted for carefully controlled feed streams to process equipment. The pump capacities and energy costs are tabulated below by fluid being pumped, with pumps for drug substance or drug product product streams (cells and antigen) in Table 3.6.1-2, additions to bioreactors (media and baculovirus) in Table 3.6.1-3, and buffers in Table 3.6.1-4. Some pumps providing media to the bioreactors have two settings: a quicker one to refill the tank with a baseline amount of media before the start of each batch, and the slower fed-batch mode setting. Settings were chosen such that the same pump and tubing size could be used without exceeding a flow velocity of 5.0 meters per second.

Table 3.6.1-2 Overview of Peristaltic Pumps for Drug Substance/Product

Equipment Tag	Flow Rate (L/hr)	Operating Time per Use (hr)	Power (W)	Operating Time (hr/year)	Energy Use (kWh/year)	Cost of Operation (USD/year)
P102	800	0.3	23.73	4.5	0.11	0.01
P201	4291	2.0	98.01	71.1	6.97	0.70
P202	6300	1.3	278.05	47.2	13.13	1.33
P203	6300	1.3	220.07	47.2	10.39	1.05
P204	6300	1.3	281.34	47.2	13.28	1.34
P205	6300	1.1	220.07	41.1	9.05	0.91
P206	400	14.1	18.92	507.2	9.59	0.97
P207	599	2.6	20.04	94.0	1.88	0.19
P208	599	2.6	20.82	94.0	1.96	0.20
P209	130	6.6	15.33	237.5	3.64	0.37
P210	130	6.6	3.36	237.5	0.80	0.08
P211	262	15.0	12.41	540.0	6.70	0.68
P212 a & b	1618	1.0	56.55	36.0	2.04	0.21
P213 a & b	17	163.4	0.38	1470.6	0.55	0.06

Table 3.6.1-2 Overview of Peristaltic Pumps for Bioreactor Additives

Equipment Tag	Substance	Flow Rate (L/hr)	Operating Time per Use (hr)	Power (W)	Operating Time (hr/yr)	Energy Use (kWh/yr)	Cost of Operation (USD/yr)
P113	Media	1.4	81.0	0.03	729.0	0.03	< 0.01
		136.0	0.5	4.12	4.1	0.02	< 0.01
P114	Media	10.8	81.0	0.32	729.0	0.23	0.02
		142.0	6.5	4.98	58.5	0.29	0.03
P115	Media	86.5	81.0	3.22	729.0	2.35	0.24
		125.0	24.0	4.91	216.0	1.06	0.11
P116	Media	34541.0	1.3	1199.26	11.3	13.49	1.36
P117-P120 a & b	Media	55.6	54.0	1.96	486.0	0.95	0.10
P121a-P124a	Baculovirus	400.0	0.2	13.98	4.4	0.06	0.01
P121b-P124b	Baculovirus	400.0	0.2	13.98	4.4	0.06	0.01

Table 3.6.1-3 Overview of Peristaltic Pumps for Buffers

Equipment Tag	Buffer	Flow Rate (L/hr)	Operating Time per Use (hr)	Power (W)	Operating Time (hr/yr)	Energy Use (kWh/yr)	Cost of Operation (USD/yr)
P214	Buffer A	10000	0.85	348.26	30.5	10.63	1.07
P215	Buffer A	6300	0.24	278.05	8.6	2.38	0.24
P219	Buffer C	6300	0.19	278.05	6.9	1.91	0.19
P220	Sanitization	6300	0.24	278.05	8.6	2.38	0.24
P216	Buffer A	6300	0.57	216.78	20.6	4.46	0.45
P217	Buffer B	6300	0.29	216.78	10.3	2.23	0.23
P218	Buffer C	6300	0.19	216.78	6.9	1.49	0.15
P221	Sanitization	6300	0.24	216.78	8.6	1.86	0.19
P223	Buffer D	400	18.00	18.92	648.0	12.26	1.24
P224	Buffer D	599	1.90	13.89	68.6	0.95	0.10
P225	Buffer E	599	1.43	13.89	51.4	0.71	0.07
P222	Sanitization	599	0.48	13.89	17.1	0.24	0.02
P226	Formulation	497	15.00	22.79	540.0	12.31	1.24
P227	Formulation	1526	0.5	70.25	18.0	1.26	0.13
P228	Storage Buffer A	6300	1.67	289.06	58.3	16.86	1.70
P229	Storage Buffer B	6300	1.67	289.06	58.3	16.86	1.70
P230	Storage Buffer A	599	3.33	27.46	116.7	3.20	0.32

3.6.2. Mixing and Holding Tanks

It is good practice to have holding tanks between major production steps in case of broken equipment or delays in the process. This facility requires eight holding tanks in upstream process after the last 10,000 L reactors and five in downstream: after each centrifugation step, after anion exchange, after hydrophobic interaction chromatography, and after the final ultrafiltration and diafiltration step. Excluding the tank after HIC, all the holding tanks will have the same dimensions of 2.78 m x 2.20 m (H x D). The eight upstream tanks and first downstream tank will require the same impeller size, baffles, and speed as the 10,000 L bioreactors to ensure the tanks are well mixed. The tank after HIC will have dimensions of 1.35 m x 1.12 m (H x D) and does not require an impeller because it is assumed to be homogenous (the same assumption applies to the other holding tanks without impellers).

Additionally, mixing tanks will be needed throughout the process to hold and prepare the buffers, media, and baculovirus. All the mixing tanks will require impellers and baffles to ensure the substances remain well mixed. The buffers, impeller numbers, and tank dimensions are summarized in Table 3.6.2-1 below. The mixing tanks for baculovirus will have impeller diameter of 0.31 m with an agitation speed of 85 min^{-1}, the tanks for media and buffer A will have impeller diameters of 0.84 m with an agitation speed of 30 min^{-1}, and the rest of the mixing tanks will have impeller diameters of 0.46 with an agitation speed of 60 min^{-1}.

Table 3.6.2-1. Mixing Tank Dimensions

Equipment Tag	Substance	Liquid Volume Required (L)	Tank Volume (L)	Tank Height (m)	Tank Diameter (m)	Number of Impellers
T101	Media	79,970	83,300	2.4	6.4	4
T102 T103	Baculovirus (x2)	780	1,250	1.35	1.12	2
T201	Buffer A	10,404	12,000	2.78	2.20	2
T202	Buffer B	2,100	2,500	1.65	1.28	2
T203	Buffer C	2,400	3,500	3.35	1.17	3
T204	Buffer D	2,964	3,500	3.35	1.17	3
T205	Buffer E	1,140	2,500	1.65	1.28	2
T206	Sanitization Buffer	3,285	3,500	3.35	1.17	3
T207	Formulation Buffer	3,000	3,500	3.35	1.17	3
T208	Storage Buffer A	1257	2,500	1.65	1.28	2
T209	Storage Buffer B	1200	2,500	1.65	1.28	2

3.6.3. Cooling Requirements

Since heat is a byproduct of both cellular metabolism and mechanical mixing with impellers, our fed-batch bioreactors require sufficient cooling to maintain a constant temperature of 27 C during their operation. To achieve this specification, cooling jackets filled with a 50% ethylene glycol/water mixture will be installed on the exterior of the reactors to facilitate heat

transfer. The cooling jackets were modeled as shell and tube heat exchangers, and the theoretical area of each jacket was calculated using Equation 3.6.3-1.

$$A_{req} = \frac{Q}{U_o * \Delta T_{lm}} \qquad \textbf{Equation 3.6.3-1}$$

Q, the amount of heat generated by the fermenters, is described in Equation 3.6.3-2 and can be found by adding the heat generated by the cells to the heat produced by the rotation of the impeller. The maximum oxygen uptake rate of each reactor, Q_{O2max}, was calculated based on the assumed specific oxygen uptake rate of Sf9 cells which was approximated to be 0.198 mmol O_2 per g biomass per hour (see Section 3.3.5). The Q_{O2max} term was multiplied by 0.12 to get the cell heat generation (Shuler & Kargi 2002). P_g represents the gassed power to the system, and its calculation is described in detail in Section 3.3.5. Table 3.6.3-1 lists the heat generated by all of the fed-batch reactors. It was assumed that the shake flasks, F101 and F012a - F102f, produce negligible heat that is effectively dissipated without intervention.

$$Q = 0.12 Q_{O_2 max} + P_g \qquad \textbf{Equation 3.6.3-2}$$

Table 3.6.3-1. Reactor Heat Generation

Equipment Tag	Working Volume (L)	Q_{O2max} (g/L*h)	Heat From Cells (W)	Gassed Power (W)	Total Heat (W)
R101	200	0.0184	0.44	23.8	24.2
R102	2000	0.0147	3.53	46.5	50.0
R103	12000	0.0196	28.23	554.9	583.1
R104a - R107a R104b - R107b	10000	0.0165	19.76	487.2	507.0

The log mean temperature difference, ΔT_{lm}, was calculated using Equation 3.6.3-3 and was found to be 3.99. T_1 represents the inlet temperature of the coolant (20 C), T_2 is the outlet temperature of the coolant (25 C), and T_H is the temperature inside the reactor (27 C).

$$\Delta T_{lm} = \frac{T_2 - T_1}{ln(\frac{T_H - T_1}{T_H - T_2})} \qquad \textbf{Equation 3.6.3-3}$$

The overall heat transfer coefficient, Uo, was calculated using Equation 3.6.3-4. The outer tank radius, r_o, and the inner tank radius, r_i, were calculated for each reactor assuming a tank wall thickness of 0.02 m. The thermal conductivity of steel, k_{steel}, was assumed to be 16.3 W/m*K.

$$U_o = (\frac{1}{h_o} + \frac{r_o}{k_{steel}} * ln(\frac{r_o}{r_i}) + \frac{1}{h_i} * \frac{r_o}{r_i})^{-1} \qquad \textbf{Equation 3.6.3-4}$$

The convective heat transfer of the coolant, h_o, was calculated using Equations 3.6.3-5 - 3.6.3-8. Table 3.6.3-2 shows the assumed constants for the coolant that were used for these calculations including its thermal conductivity, linear velocity through the jacket piping, specific heat capacity, viscosity, and density. Table 3.6.3-3 shows the results of these calculations for each reactor.

$$h_o = \frac{Nu * k_{glycol}}{D_{tube}} \qquad \textbf{Equation 3.6.3-5}$$

$$Nu = 0.023 * Re^{0.8} * Pr^{0.4} \qquad \textbf{Equation 3.6.3-6}$$

$$Re = \frac{v * p * D_{tube}}{\mu} \qquad \textbf{Equation 3.6.3-7}$$

$$Pr = \frac{C_p * \mu}{k_{glycol}} \qquad \textbf{Equation 3.6.3-8}$$

Table 3.6.3-2. Coolant Constants (at 20 C)

Constant	Units	Value
k_{glycol}	(W/m*K)	0.41
v	(m/s)	1.25
C_p	(kJ/kg*K)	3.50
μ	(Pa*s)	0.0042
ρ	(kg/m³)	1060

Table 3.6.3-3. Relevant Coolant Parameters

Equipment Tag	Working Volume (L)	D_{tube} (m)	h_o (W/m²K)	Nu	Re	Pr
R101	200	0.01	156.85	3.83	3154	0.036
R102	2000	0.01	156.85	3.83	3154	0.036
R103	12000	0.01	156.85	3.83	3154	0.036
R104a - R107a R104b - R107b	10000	0.01	156.85	3.83	3154	0.036

The convective heat transfer of the cell broth, h_i, was calculated using Equations 3.6.3-9 - 3.6.3-11. Table 3.6.3-4 shows the assumed constants for the cell broth that were used for these calculations including its thermal conductivity, specific heat capacity, bulk viscosity at 27° C, tank wall interface viscosity at 20 C (assumed to be at the inlet temperature of the coolant), and density. Table 3.6.3-5 shows the results of these calculations for each reactor.

$$h_i = k_{water} * a * Re^b * Pr^{1/3} * \left(\frac{\mu}{\mu_s}\right)^{m'} * \frac{1}{D_T} \qquad \textbf{Equation 3.6.3-9}$$

$$Re = \frac{D_i^2 * N * \rho}{\mu} \qquad \textbf{Equation 3.6.3-10}$$

$$Pr = \frac{C_p * \mu}{k_{water}}$$ **Equation 3.6.3-11**

Table 3.6.3-4. Cell Broth Constants

Constant	Units	Value
a	-	0.54
b	-	0.67
m’	-	0.14
k_{water}	(W/m*K)	0.62
C_p	(kJ/kg*K)	4.18
μ	(Pa*s)	0.00085
μ_s	(Pa*s)	0.00113
ρ	(kg/m³)	1031

Table 3.6.3-5. Relevant Cell Broth Parameters

Equipment Tag	Working Volume (L)	$D_{impeller}$ (m)	h_i (W/m²K)	N (rad/s)	Re	Pr
R101	200	0.31	1043	10.16	1183870	0.0057
R102	2000	0.46	501	4.61	1167062	0.0057
R103	12000	0.89	560	3.56	3412648	0.0057
R104a - R107a R104b - R107b	10000	0.84	571	3.77	3203041	0.0057

The actual area of each cooling jacket was found using Equation 3.6.3-12, where h_T is the wetted height of the tank and D_T is the diameter of the tank. All of the calculated required areas per Equation 3.6.3-1 are smaller than the actual areas, meaning that this heat design is sufficient

to cool the reactor. Equation 3.6.3-13 was used to calculate the mass flow of the coolant, and this mass flow was converted into a volumetric flow rate using Equation 3.6.3-14. Table 3.6.3-6 summarizes the cooling requirements of our process.

$$A_{jacket} = \pi h_T D_T \qquad \textbf{Equation 3.6.3-12}$$

$$m_c = \frac{Q}{(T_2 - T_1)C_p} \qquad \textbf{Equation 3.6.3-13}$$

$$V = \frac{m_c}{\rho} \qquad \textbf{Equation 3.6.3-14}$$

Table 3.6.3-6. Summary of Cooling Requirements

Equipment Tag	Working Volume (L)	U_o (W/m^2K)	Actual Jacket Area (m^2)	Required Jacket Area (m^2)	Coolant Flow Rate (L/hr)	Total Coolant Per Cycle (L)
R101	200	115.4	1.29	0.053	0.96	78
R102	2000	103.3	6.25	0.121	1.98	161
R103	12000	106.1	20.54	1.377	23.11	1872
R104a - R107a R104b - R107b	10000	106.4	18.18	1.194	20.09	2089

3.6.4. CIP & SIP

Ease of operation and reduction of hazardous materials held on site were held paramount in the development of the clean in place (CIP) and sanitize in place (SIP) procedures for the facility. For this reason, the majority of equipment selected for this process is single-use. For example, all filters, peristaltic piping, and tank liners are disposed of after every use. Although this produces large amounts of solid waste, it reduces the environmental and occupational hazards presented by the presence of large amounts of caustic on site.

By contrast, the high equipment costs of the disc-stack centrifuges and chromatography resins require that they are cleaned and reused. The centrifuges are cleaned by hand by trained personnel between uses, following proper Lock Out Tag Out (LOTO) procedures. The chromatography columns are regenerated, cleaned, sanitized, and stored in their respective buffers for reuse. The environmental and economic effects of these cleaning and sanitization design decisions are elaborated upon in Section 6.

4. Final Design

4.1. Upstream

4.1.1. Preseason Expansion

The seed train for our process begins in the offseason, where initial cell expansion occurs in a 5 L working volume master shake flask (F101). Sf9 cells are seeded at a density of 2.44 x 10^5 cells/mL in 5 L of growth media and incubated at 27 °C for 81 hrs. The cells reach a maximum density of 1.95 x 10^6 cells/mL and are expanded into six additional 5 L working volume shake flasks (F102a - F102f) at an initial density of 3.26 x 10^5 cells/mL (See Upstream Process Flow Diagram, Figure 3.1-1, Page 8). The cells are once again incubated at 27 °C for 81 hrs until each flask reaches a density of 2.60 x 10^6 cells/mL.

The contents of F102a - F102f are then transferred to a 200 L working volume stirred tank reactor (R101). This reactor has a height of 0.701 m, a diameter of 0.620 m, and is fitted with four baffles, each with a diameter of 0.180 m. Its contents are stirred by a marine impeller with a diameter of 0.310 m and an operating speed of 97 rpm. Initially, 61 L of growth media is added to the reactor from mixing tank T101 along with the seed stock. This starting condition corresponds to a 3.91 x 10^5 cells/mL seeding density. The cells are grown for 81 hrs at 27 °C to a final density of 3.13 x 10^6 cells/mL. During this growth period, media is continuously supplied from T101 at a constant rate of 1.35 L/hr. Air is also sparged into the reactor from a gas line at a constant rate of 2418 L/hr. The reactor's temperature is maintained using a 1.29 m² cooling jacket, which circulates glycol coolant at 0.96 L/hr, entering at 20 °C and exiting at 25 °C. At the end of the growth period, the reactor's contents are sent to a freezer (FR102) and stored until the start of the production season. The preseason expansion process is repeated eighteen times in the

offseason to generate the frozen starting stock needed for the nine batches produced throughout the production season.

4.1.2. Production Fermentation

To initiate production, 200 L of seed stock produced by R101 (see Upstream Process Flow Diagram, Figure 3.1-1, Page 8) is thawed and transferred to R102, a 2000 L working volume stirred tank reactor, along with 924 L of growth media supplied by T101. This reactor has a height of 1.651 m, a diameter of 1.280 m, and is fitted with four baffles, each with a diameter of 0.130 m. Its contents are stirred by two marine impellers, each with a diameter of 0.457 m and an operating speed of 44 rpm. The seeding density is 3.13×10^5 cells/mL. The cells are grown for 81 hrs at 27 °C to a final density of 2.50×10^6 cells/mL. During this growth period, media is continuously supplied from T101 at a constant rate of 10.81 L/hr. Air is also sparged into the reactor from a gas line at a constant rate of 3528 L/hr. The reactor's temperature is maintained using a 6.25 m² cooling jacket, which circulates glycol coolant at 1.98 L/hr, entering at 20 °C and exiting at 25 °C. At the end of the growth period, the contents of R102 are transferred to R103.

R103 is a 12000 L working volume stirred tank reactor. It has a height of 2.945 m, a diameter of 2.337 m, and is fitted with four baffles, each with a diameter of 0.203 m. Its contents are stirred by two marine impellers, each with a diameter of 0.889 m and an operating speed of 36 rpm. The seed stock from R102 and 2993 L of media from T101 are initially added to the reactor, corresponding to a seeding density of 4.17×10^5 cells/mL. The cells are grown for 81 hrs at 27 °C to a final density of 3.33×10^6 cells/mL. During this growth period, media is continuously supplied from T101 at a constant rate of 86.51 L/hr. Air is also sparged into the reactor from a gas line at a constant rate of 20066 L/hr. The reactor's temperature is maintained

using a 20.54 m² cooling jacket, which circulates glycol coolant at 23.11 L/hr, entering at 20 °C and exiting at 25 °C. At the end of the growth period, the contents from R103 are split between eight infection reactors, R104a - R107a and R104b - R107b.

R104a - R107a and R104b - R107b are 10000 L working volume stirred tank reactors. They have a height of 2.772 m, a diameter of 2.200 m, and are fitted with four baffles, each with a diameter of 0.169 m. Each reactor's contents are stirred by two marine impellers, each with a diameter of 0.837 m and an operating speed of 34 rpm. Each infection reactor is initially filled with 1500 L of seed stock from R103 along with 5397 L of media from T101, corresponding to a seeding density of 5.00 x 10^5 cells/mL. The cells are grown for 54 hrs at 27 °C to a final density of 2.00 x 10^6 cells/mL. During this growth period, media is continuously supplied from T101 at a constant rate of 55.61 L/hr. At the end of the growth period, mixing tank T102 delivers 400 L of baculovirus stock solution, with a concentration of 10^5 PFU/L (MOI of 1), to each type A reactor (R104a - R107a), infecting the Sf9 cells for type A antigen production. Similarly, mixing tank T103 supplies the same volume and concentration of baculovirus stock solution to each type B reactor (R104b - R107b). Once the cells are infected, they produce antigen for 50 hrs and subsequently self-lyse. Each reactor yields 0.20 kg of antigen. Throughout the growth and infection periods, air is sparged into each reactor at a constant rate of 17732 L/hr, providing the necessary oxygen for cell metabolism and accounting for the 40% increase in oxygen consumption that occurs post-infection (Gotoh et al., 2004). Each reactor's temperature is maintained using a 18.18 m² cooling jacket, which circulates glycol coolant at 20.09 L/hr, entering at 20 °C and exiting at 25 °C.

The contents of each infection reactor are then transferred to mixing tanks H101a - H104a and H101b - H104b, which serve as holding tanks before the cell slurry moves into the

downstream purification train. Each holding tank has a 10000 L working volume, with dimensions and impeller specifications identical to those of the infection fermenters. The production fermentation process is repeated twice per batch, totaling eighteen times each production season.

4.2. Downstream

4.2.1. Extraction Centrifugation

The cell slurry from one set of holding tanks (H101a - H104a or H101b - H104b) is then sent through extraction centrifugation to separate the spent media, resulting in a cell pellet that contains the lysed Sf9 cells and the target antigen (see Downstream Process Flow Diagram, Figure 3.1-2, Page 9). To handle the 40000 L of incoming cell slurry, four extraction centrifuges (C201 - C204) are used. These centrifuges are Westfalia Type Disk Stack Centrifuge Separators, with 110 disks angled at 45 degrees. The outer disk radius is 0.977 m, while the inner disk radius is 0.275 m. Each centrifuge operates at 4484 rpm, processing 10000 L of broth at a rate of 374.25 L/hr, leading to a processing time of 26.72 hrs. This extraction process achieves a 98% cell yield and a 95% antigen yield, resulting in a total of 72.60 kg of cells and 0.74 kg of antigen exiting this step. The cumulative waste stream contains 1.48 kg of cells and 0.06 kg of antigen diluted in 40000 of spent media.

The cell pellet is subsequently transferred to baffled mixing tank H201, where it is resuspended in 8487 L of Buffer A supplied from buffer holding tank T201. Including the volume of the cells, the total volume of the resuspension is 8708 L. This mixture is then sent to C205 to undergo clarification centrifugation.

4.2.2. Clarification Centrifugation

C205 is a two-phase disk stack separator, model GNLD-40 from GN Separation, with 40 disks angled at 45 degrees. The outer disk radius is 0.44 m, while the inner disk radius is 0.15 m. Operating at 3280 rpm, it processes the 8708 L of resuspension at a rate of 4291 L/hr, leading to a processing time of 2.03 hrs. This centrifuge separates the lysed cells from the target antigen, with the cells settling due to the centrifugal forces while the antigen remains in the supernatant. It achieves 100% cell clearance and 95% antigen recovery, with 0.71 kg of antigen remaining in the supernatant which consists of 8488 L of Buffer A. The waste from this step is a 72.60 kg cell pellet containing 0.04 kg of antigen. The supernatant is transferred to holding tank H202 before proceeding to the next purification step.

4.2.3. Anion Exchange Chromatography

The clarified antigen suspension then undergoes anion exchange chromatography, where the target antigen is separated from negatively charged genetic material and native cell proteins. AXR201, the anion exchange column, has a length of 0.20 m, a diameter of 1.38 m, and a volume of 300 L. It is packed with positively charged Cytiva Capto Q ImpRes resin and is operated at 20 °C and 0.47 bar pressure. To maintain this low operating pressure, material is flowed through the column at 6300 L/hr. At the beginning of a cycle, the column is first equilibrated with 5 column volumes (CVs) of Buffer A, supplied by T201. Then, 7.22 CVs of the antigen suspension are loaded onto the column from H202. AXR201 is operated in flowthrough mode, with purified antigen in Buffer A exiting the column and being collected. Waste proteins are then eluted off the column with 4 CVs of Buffer C, delivered by T203. The column is sanitized with 5 CVs of a buffer containing 1 M NaCl and 0.5 M NaOH, supplied by T204, and

is subsequently regenerated with 5 CVs of WFI. It is finally re-equilibrated with Buffer A to begin the next loading cycle. Each cycle lasts approximately 1.25 hours, and four cycles are required to process the entire antigen suspension, resulting in a total processing time of 5 hours. When not in use, this column is stored in 1 CV of a 20% ethanol solution. AXR201 achieves a 99.99% antigen recovery, so 0.71 kg of antigen in 8488 L of Buffer A leaves this step and is temporarily transferred to holding tank H203. The waste stream contains 6000 L of Buffer A, 4800 L of Buffer C, 6000 L of sanitization buffer, 6000 L of WFI, 1200 L of storage buffer, and the negatively charged waste proteins.

4.2.4. Cation Exchange Chromatography

The next step in the purification process is cation exchange chromatography (CXR201), where the target antigen is separated from any positively charged cell debris. CXR201, the cation exchange column, has identical dimensions to AXR201. It is packed with negatively charged Cytiva Capto SP ImpRes resin and is operated at 20 °C and 0.47 bar pressure. To maintain this low operating pressure, material is flowed through the column at 6300 L/hr. At the beginning of a cycle, the column is first equilibrated with 5 CVs of Buffer A, supplied by T201. Then, 7.22 CVs of the antigen suspension are loaded onto the column from H203. CXR201 is operated in bind-and-elute mode, meaning that the target antigen binds to the resin while the impurities flow through the column. To ensure none of the impurities remain in the column, it is washed with an additional 7 CVs of Buffer A from T201 after loading. Next, the target antigen is eluted from the column with 6 CVs of Buffer B supplied by T202, and this eluate is collected. Any leftover impurities bound to the column are then eluted using 4 CVs of Buffer C delivered by T203. Finally, the column is sanitized with 5 CVs of sanitization buffer from T204 and regenerated

with 5 CVs of WFI before being re-equilibrated with Buffer A for the next loading cycle. Each cycle lasts approximately 1.87 hours, and four cycles are required to process the entire antigen suspension, resulting in a total processing time of 7.5 hours. When not in use, this column is stored in 1 CV of a 20% ethanol solution with 0.2 M sodium acetate. CXR201 achieves a 99.99% antigen recovery, so 0.71 kg of antigen in 7200 L of Buffer B leaves this step and is transferred to UF201, an ultrafiltration and diafiltration system. The waste stream contains 22888 L of Buffer A, 4800 L of Buffer C, 6000 L of sanitization buffer, 6000 L of WFI, 1200 L of storage buffer, and the positively charged impurities.

4.2.5. Ultrafiltration and Diafiltration

UF201 is a tangential flow filtration system fitted with three hollow fiber filter cartridges from Cytiva. These filters have a surface area of 6 m^2, a protein rejection coefficient of 0.99, and a buffer rejection coefficient of 0.00. The system is first operated in ultrafiltration mode to concentrate the eluate from CXR201 from 7200 L to 1563 L. To accomplish this, the system circulates the eluate while maintaining a permeate flux of 6.17 x 10^{-6} m/s, and it takes 14.09 hrs for the solution to become fully concentrated. The system is then switched to diafiltration mode to exchange Buffer B for Buffer D, ensuring compatibility with the next chromatography step. During operation, Buffer D is continuously supplied from T205, and the permeate flux through the filters is maintained at the same rate as in ultrafiltration mode. It takes 7200 L of Buffer D and 18 hrs to complete the exchange, resulting in a total processing time of approximately 32 hrs. UF201 achieves a 94.09% recovery of the target antigen, so 0.66 kg antigen in 1563 L of Buffer D leaves this step. The waste stream contains 7200 L of Buffer B, 5637 L of Buffer D, and 0.05 kg of the antigen.

4.2.6. Hydrophobic Interaction Chromatography

The product is then sent to HIC201, a hydrophobic interaction chromatography column that serves as a polishing step to remove any aggregates from the target antigen monomer. It has a length of 0.20 m, a diameter of 0.60 m, and a volume of 57 L. It is packed with Cytiva Capto Butyl ImpRes resin and is operated at 20 °C and 0.21 bar pressure. To maintain this low operating pressure, material is flowed through the column at 598.5 L/hr. At the beginning of a cycle, the column is first equilibrated with 10 column volumes (CVs) of Buffer D, supplied by T205. The resin's binding capacity is high enough to accommodate the entire 1563 L of solution exiting UF201, so it is loaded all at once, corresponding to 27.42 CVs. HIC201 operates in bind-and-elute mode, meaning that the target monomer binds strongly to the resin, while the unwanted aggregates flow through. To ensure none of the aggregates remain in the column, it is washed with an additional 10 CVs of Buffer D from T205 after loading. Next, the target antigen is eluted from the column with 15 CVs of Buffer E supplied by T206, and this eluate is collected. Finally, the column is sanitized with 5 CVs of sanitization buffer from T204 and regenerated with 5 CVs of WFI. As previously stated, each run of HIC201 requires only one cycle, and each cycle takes 6.9 hrs. When not in use, this column is stored in 1 CV of a 20% ethanol solution. HIC201 achieves a 92% recovery of the target antigen, so 0.61 kg of antigen in 855 L of Buffer E leaves this step and is temporarily transferred to holding tank H204. The waste stream contains 2703 L of Buffer D, 285 L of sanitization buffer, 285 L of WFI, 57 L of storage buffer, the antigen aggregates, and 0.05 kg of the target antigen.

4.2.7. Viral Filtration

The final separation step of the purification process is viral filtration, where any remaining baculovirus particles are removed from the product. Viral filtration system VF201 consists of a 6 m^2 Cytiva Ultipor™ VF grade DV20 filter - a 160 kDa molecular weight cut-off (MWCO) membrane designed to remove particles larger than the target antigen (63 kDa). The antigen solution is discharged from H204 and is passed through the filter at a flow rate of 129.6 L/hr, corresponding to a permeate flux of 2.16 x 10^{-2} m/s and an operating pressure of 2.10 bar. The total processing time to filter all 855 L of antigen solution is 6.6 hrs. VF201 achieves a 99% antigen recovery, so 0.61 kg of antigen in 855 L of Buffer E leaves this step. There is no waste stream, as the negligible amount of virus that may remain in the filter does not reduce the solution's volume.

4.2.8. Final Diafiltration

The purified antigen solution exiting VF201 is transferred to UF202, where Buffer E is exchanged for the final formulation buffer. UF202 is identical to UF201, but it is solely operated in diafiltration mode. During operation, the system circulates the solution while maintaining a permeate flux of 4.05 x 10^{-6} m/s. The formulation buffer is continuously supplied from T207, and it takes approximately 3937 L and 15 hrs to complete the exchange. UF202 achieves a 97% recovery of the target antigen, so 0.59 kg of antigen in 855 L of formulation buffer leaves this step and is transferred to mixing tank H205. The waste stream contains 855 L of Buffer E, 3082 L of formulation buffer, and 0.02 kg of the target antigen.

4.3. Formulation and Filling

The final drug substance is formulated in H205, where an additional 763 L of formulation buffer, supplied by T207, is added to the mixing tank along with the 855 L of antigen solution coming from UF202. The entire downstream process is repeated four times to yield a final solution containing two type A antigens and two type B antigens, totaling 2.36 kg of antigen in approximately 6472 L of solution per batch. This solution is then sent to FF201a and FF201b, two aseptic filling lines capable of filling 600 vials/min. The filling lines operate in parallel, enabling the filling of 72000 vials per hour. As a result, each batch takes about one week to complete. FF201a and FF201b achieve a yield of 85%, accounting for 10% product loss in pipes during startup and shutdown, and 5% loss due to packaging errors. The filling workcell is programmed to fill each single-dose vial with 0.55 mL of the final formulation, which includes a 10% overfill. In total, approximately 61 days are required to fill 90,000,163 vials across nine batches, surpassing our production goal of 90 million doses per production season. The extra doses are retained for quality control purposes.

4.4. Ancillary Equipment

4.4.1. Pumps

There are 79 pumps included in the proposed design: 26 compressors and 53 peristaltic pumps. Each will be operated with shear and efficiency in mind, minimizing damage to product without causing major production delays. The total energy cost of operating all pumps for one production season is USD $1,784.

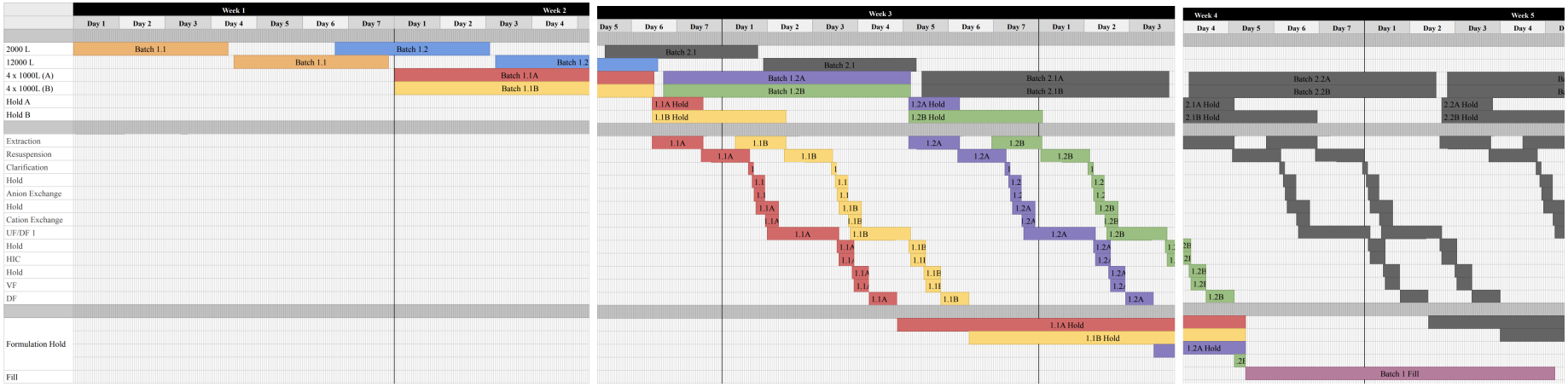
4.4.2 Mixing and Holding Tanks

This process requires 13 holding tanks between the major steps of the upstream and downstream trains and 12 mixing tanks to prepare buffers, media, and virus. The dimensions and impeller specifications were determined using similar values to the bioreactors for consistency. The location of these tanks will be determined by carefully evaluating the safety hazard of each substance.

4.5. Production Scheduling

Manufacturing an influenza vaccine is uniquely challenging due to the rapid evolution of the virus. The nature of the virus requires flexibility and rapid reaction times in the manufacturing process. This requirement is fulfilled by the proposed process, which delivers 90 million doses in 18 weeks. A fraction of the proposed schedule is included in full in Figure 4.5-1 below, detailing one of nine production batches in color, with succeeding batches shown in greyscale.

Figure 4.5-1. Production Schedule



4.6. Complete Stream Table

Upstream

Stream Number	Location	Contents	Flow Rate		Flow Rate	
0	F102a - F102f R101 In	Cell Broth	30.00	L/run	60.00	L/batch
		Cells	0.07	kg/run	0.14	kg/batch
		Air	195858.00	L/run	391716.00	L/batch
1	R101 Out FR102 In	Cell Broth	200.00	L/run	400.00	L/batch
		Cells	0.58	kg/run	1.16	kg/batch
		Air	0.00	L/run	0.00	L/batch
2	FR102 Out R102 In	Cell Broth	200.00	L/run	400.00	L/batch
		Cells	0.58	kg/run	1.16	kg/batch
		Air	285736.41	L/run	571472.82	L/batch
3	R102 Out R103 In	Cell Broth	2000.00	L/run	4000.00	L/batch
		Cells	4.63	kg/run	9.26	kg/batch
		Air	1625356.02	L/run	3250712.05	L/batch
4	R103 Out R104a - R107a In R104b - R107b In	Cell Broth	12000.00	L/run	24000.00	L/batch
		Cells	37.04	kg/run	74.08	kg/batch
		Air	14753038.89	L/run	29506077.78	L/batch
5a - 8a (Per Stream)	R104a - R107a Out H101a - H104a In	Cell Broth	10000.00	L/run	20000.00	L/batch
		Cells	18.52	kg/run	37.04	kg/batch
		Antigen	0.20	kg/run	0.39	kg/batch
		Air	0.00	L/run	0.00	L/batch
5b - 8b (Per Stream)	R104b - R107b Out H101b - H104b In	Cell Broth	10000.00	L/run	20000.00	L/batch
		Cells	18.52	kg/run	37.04	kg/batch
		Antigen	0.20	kg/run	0.39	kg/batch
		Air	0.00	L/run	0.00	L/batch
9a - 12a (Per Stream)	H101a - H104a Out C201 - C204 In	Cell Broth	10000.00	L/run	20000.00	L/batch
		Cells	18.52	kg/run	37.04	kg/batch
		Antigen	0.20	kg/run	0.39	kg/batch
9b - 12b (Per Stream)	H101b - H104b Out C201 - C204 In	Cell Broth	10000.00	L/run	20000.00	L/batch
		Cells	18.52	kg/run	37.04	kg/batch
		Antigen	0.20	kg/run	0.39	kg/batch

Stream Number	Location	Contents	Flow Rate		Flow Rate	
13	T101 Out R101 In	Media (Hink's TNM-FH)	170.00	L/run	340.00	L/batch
14	T101 Out R102 In	Media (Hink's TNM-FH)	1800.00	L/run	3600.00	L/batch
15	T101 Out R103 In	Media (Hink's TNM-FH)	10000.00	L/run	20000.00	L/batch
16	T101 Out R104a - R107a In R104b - R107b In	Media (Hink's TNM-FH)	67200.00	L/run	134400.00	L/batch
17	T102 Out R104a - R107a In	Baculovirus Stock Solution (10^{11} PFU/L)	400.00	L/run	800.00	L/batch
18	T103 Out R104b - R107b In	Baculovirus Stock Solution (10^{11} PFU/L)	400.00	L/run	800.00	L/batch

Downstream

Stream Number	Location	Contents	Flow Rate		Flow Rate	
19-22 (Total)	C201 - C204 Out H201 In	Cell Broth	0.00	L/run	0.00	L/batch
		Cells	72.60	kg/run	290.39	kg/batch
		Antigen	0.74	kg/run	2.96	kg/batch
23	H201 Out C205 In	Buffer A	8487.72	L/run	33950.88	L/batch
		Cells	72.60	kg/run	290.39	kg/batch
		Antigen	0.74	kg/run	2.96	kg/batch
24	C205 Out H202 In	Buffer A	8487.72	L/run	33950.88	L/batch
		Cells	0.00	kg/run	0.00	kg/batch
		Antigen	0.71	kg/run	2.82	kg/batch
25	H202 Out AXR201 In	Buffer A	8487.72	L/run	33950.88	L/batch
		Antigen	0.71	kg/run	2.82	kg/batch
26	AXR201 Out H203 In	Buffer A	8487.72	L/run	33950.88	L/batch
		Antigen	0.71	kg/run	2.82	kg/batch
27	H203 Out CXR201 In	Buffer A	8487.72	L/run	33950.88	L/batch
		Antigen	0.71	kg/run	2.82	kg/batch
28	CXR201 Out UF201 In	Buffer B	7200.00	L/run	28800.00	L/batch
		Antigen	0.71	L/run	2.84	L/batch
29	UF201 Out (Waste)	Buffer B	7200	L/run	28800	L/batch
		Buffer D	5636.54	L/run	22546.16	L/batch
		Antigen	0.05	kg/run	0.19	kg/batch
30	UF201 Out HIC201 In	Buffer D	1563.46	L/run	6253.84	L/batch
		Antigen	0.66	kg/run	2.65	kg/batch
31	HIC201 Out H204 In	Buffer E	855.00	L/run	3420.00	L/batch
		Antigen	0.61	kg/run	2.44	kg/batch
32	H204 Out VF201 In	Buffer E	855.00	L/run	3420.00	L/batch
		Antigen	0.61	kg/run	2.44	kg/batch
33	VF201 Out UF202 In	Buffer E	855.00	L/run	3420.00	L/batch
		Antigen	0.61	kg/run	2.44	kg/batch
34	UF202 Out (Waste)	Buffer E	855.00	L/run	3420.00	L/batch
		Formulation Buffer	3082.42	L/run	12329.68	L/batch
		Antigen	0.02	kg/run	0.08	kg/batch

Stream Number	Location	Contents	Flow Rate		Flow Rate	
35	UF202 Out H205 In	Formulation Buffer	855.00	L/run	3420.00	L/batch
		Antigen	0.59	kg/run	2.36	kg/batch
36a - 36b (Total)	H205 Out FF201a - FF201b	Formulation Buffer	1617.65	L/run	6470.60	L/batch
		Antigen	0.59	kg/batch	2.36	kg/batch
37	T201 Out H201 In	Buffer A	8487.72	L/run	33950.88	L/batch
38	T201 Out AXR201 In	Buffer A	6000.00	L/run	24000.00	L/batch
39	T201 Out CXR201 In	Buffer A	14400.00	L/run	57600.00	L/batch
40	T202 Out CXR201 In	Buffer B	7200.00	L/run	28800.00	L/batch
41	T203 Out CXR201 In	Buffer C	4800.00	L/run	19200.00	L/batch
42	T203 Out AXR201 In	Buffer C	4800.00	L/run	19200.00	L/batch
43	T204 Out AXR201 In	Sanitization	6000.00	L/run	24000.00	L/batch
44	T204 Out CXR201 In	Sanitization	6000.00	L/run	24000.00	L/batch
45	T204 Out HIC201 In	Sanitization	285.00	L/run	1140.00	L/batch
46	T205 Out UF201 In	Buffer D	7200.00	L/run	28800.00	L/batch
47	T205 Out HIC201 In	Buffer D	1140.00	L/run	4560.00	L/batch
48	T206 Out HIC201 In	Buffer E	855.00	L/run	3420.00	L/batch
49	T207 Out UF202 In	Formulation Buffer	3937.42	L/run	15749.68	L/batch
50	T207 Out H205 In	Formulation Buffer	762.65	L/run	3050.60	L/batch
51	T208 Out AXR201 In	Storage A	1200.00	L/run	4800.00	L/batch
52	T209 Out CXR201 In	Storage B	1200.00	L/run	4800.00	L/batch

Stream Number	Location	Contents	Flow Rate		Flow Rate	
53	T208 Out HIC201 In	Storage A	57.00	L/run	228.00	L/batch
54-57 (Total)	C201 - C204 Out (Waste)	Cell Broth	40000.00	L/run	160000.00	L/batch
		Cells	1.48	kg/run	5.93	kg/batch
		Antigen	0.06	kg/run	0.24	kg/batch
58	C205 Out (Waste)	Cells	72.60	kg/run	290.40	kg/batch
		Antigen	0.04	kg/run	0.14	kg/batch
59	AXR201 Out (Waste)	Buffer A	6000.00	L/run	24000.00	L/batch
		Buffer C	4800.00	L/run	19200.00	L/batch
		Sanitization	6000.00	L/run	24000.00	L/batch
		WFI	6000.00	L/run	24000.00	L/batch
		Storage A	1200.00	L/run	4800.00	L/batch
		Antigen	0.00	kg/run	0.00	kg/batch
60	CXR201 Out (Waste)	Buffer A	22887.72	L/run	91550.88	L/batch
		Buffer C	4800.00	L/run	19200.00	L/batch
		Sanitization	6000.00	L/run	24000.00	L/batch
		WFI	6000.00	L/run	24000.00	L/batch
		Storage B	1200.00	L/run	4800.00	L/batch
		Antigen	0.00	kg/run	0.00	kg/batch
61	HIC201 Out (Waste)	Buffer D	2703.46	L/run	10813.84	L/batch
		Sanitization	285.00	L/run	1140.00	L/batch
		WFI	285.00	L/run	1140.00	L/batch
		Storage A	57.00	L/run	228.00	L/batch
		Antigen	0.05	kg/run	0.21	kg/batch

5. Economics

5.1 Capital Investment and Equipment Costs

5.1.1. Fixed Capital Investment

The quadrivalent recombinant influenza vaccine production facility is designed to produce 90 million doses annually in Brazil. Capital investment was estimated using the factorial method, considering both direct and indirect costs associated with facility construction and operation. Costs for equipment were determined using various sources, including vendor-listed prices for similar equipment scaled to our design specifications, as well as cost correlations from literature (Towler, G. P. & Sinnott, R.K., 2013). Purchase costs for select equipment was estimated using the following equation:

$$C_e = a + bS^n \qquad \textbf{Equation 5.1.1-1}$$

Where:

- C_e is the purchased equipment cost in 2010 in the U.S. Gulf (Chemical Engineering Plant Cost Index, CEPCI = 532.9)
- a and b are cost constants
- S is the size parameter
- n is an equipment-specific exponent.

This equation was used to estimate purchase costs of bioreactors and ancillary equipment, including tanks, holding tanks with propellers, and compressors. Constants used in these calculations are detailed in Table 11-2. A 10% markup was applied to bioreactor costs to account for sparger customizations. All calculated estimates were adjusted to reflect 2025 prices using a CEPCI of 800. The total equipment purchased cost $7,466,656. Using a Lang factor of 4.74 for

fluids processing plants and a location factor of 1.15 for production in Brazil, the installed equipment cost breakdown is provided in Table 5.1.1-1.

Table 5.1.1-1 Equipment Purchase and Installed Costs

Equipment	Equipment Tag	Purchase Cost for All Units (USD)	Installed Cost in Brazil (USD)
Cell Bank Freezers	FR101a FR101b	$860	$4,697
5 L Shake Flasks	F101 F102a F102b F102c F102d F102e F102f	$438	$2,388
Culture Incubator Shakers	IS101a IS101b IS101c IS101d	$22,486	$122,571
200 L Bioreactor	R101	$117,117	$721,614
Stock Freezer	FR102	$4,799	$26,159
2,000 L Bioreactor	R102	$196,537	$1,062,946
12,000 L Bioreactor	R103	$496,791	$2,689,296
10,000 L Bioreactors	R104a R104b R105a R105b R106a R106b R107a R107b	$3,545,967	$19,195,523
Centrifuges	C201 C202 C203 C204	$125,000	$681,375

Equipment	Equipment Tag	Purchase Cost for All Units (USD)	Installed Cost in Brazil (USD)
	C205		
Anion Exchange Column	AXR201	$156,250	$851,719
Cation Exchange Column	CXR201	$156,250	$851,719
Ultrafiltration Systems	UF201 UF202	$40,000	$218,040
Hydrophobic Interaction Chromatography Skid	HIC201	$125,000	$681,375
Viral Filtration System	VF201	$32,500	$177,158
Aseptic Filling Lines	FF201a FF201b	$1,269,547	$6,920,300
Media Tank	T101	$60,316	$328,784
Baculovirus Tanks	T102 T103	$41,491	$226,166
Production Holding Tanks	H101a H101b H102a H102b H103a H103b H104a H104b	$277,707	$1,513,782
Buffer A Tank	T201	$21,085	$114,932
First Post-Centrifuge Holding Tank	H201	$34,713	$189,223
Second Post-Centrifuge Holding Tank	H202	$34,713	$189,223

Equipment	Equipment Tag	Purchase Cost for All Units (USD)	Installed Cost in Brazil (USD)
Post-AEX Holding Tank	H203	$34,713	$189,223
Post-HIC Holding Tank	H204	$34,713	$189,223
Buffer B Tank	T202	$20,745	$113,083
Buffer C Tank	T203	$20,745	$113,083
Sanitization Buffer Tank	T204	$20,745	$113,083
Buffer D Tank	T205	$20,745	$113,083
Buffer E Tank	T206	$20,745	$113,083
Formulation Buffer Tank	T207	$20,745	$113,083
Storage Tank A	T208	$20,745	$113,083
Storage Tank B	T209	$20,745	$113,083
Final Formulation Holding Tank	H205	$34,713	$189,223
Autoclave	A101	$22,500	$122,648
Compressors	P101 P103 P104 P105a P105b P106a P106b P107a P107b P108a P108b P109 P110 P111 P112	$327,903	$1,787,297

Equipment	Equipment Tag	Purchase Cost for All Units (USD)	Installed Cost in Brazil (USD)
	P125 P126 P127 P128a P128b P129a P129b P130a P130b P131a P131b		
Peristaltic Pumps	P102 P113 P114 P115 P116 P117a P117b P118a P118b P119a P119b P120a P120b P121a P121b P122a P122b P123a P123b P124a P124b P201 P202 P203 P204 P205 P206 P207 P208 P209	$158,000	$861,258

Equipment	Equipment Tag	Purchase Cost for All Units (USD)	Installed Cost in Brazil (USD)
	P210 P211 P212a P212b P213a P213b P214 P215 P216 P217 P218 P219 P220 P221 P222 P223 P224 P225 P226 P227 P228 P229 P230		

The Inside Battery Limits (ISBL) installed capital cost is $40,823,389. Using factors outlined in Table 11-3, additional cost components include:

- Outside Battery Limits (OSBL): $16,329,355
- Design and Engineering: $12,247,017
- Contingency: $4,082,339

The land cost is estimated at $17,145,823, calculated as 2% the sum of ISBL and OSBL costs. The total Fixed Capital Investment (FCI) is $90,627,923.

5.1.2. Total Capital Investment

Working capital, calculated as 15% the sum of ISBL, OSBL, design and engineering, and contingency costs, adds $11,022,315. This results in a Total Capital Investment (TCI) of $101,650,238.

5.2 Operating Costs

5.2.1 Variable Operating Costs

Variable costs include raw materials, utilities, and waste management. Total cost of raw materials is $74,043,058 per year (Table 5.2.1-1). Waste management was estimated with all waste streams (Table 6.3-1) classified as hazardous waste and disposed of at a rate of $500 per ton, more details in section 6.3. Utilities were conservatively estimated as 10% of the sum of waste disposal and raw materials costs. The total variable operating costs are estimated at $82,898,014 annually.

Table 5.2.1-1 Raw Materials Quantities and Costs

Raw Material	Annual Quantity	Cost
Media	1,400,000 L	$1,112,980
Bioreactor Liners	180	$14,903
AEX Resin	300 L	$44,414
CEX Resin	300 L	$44,414
UF/DF Filters	27	$181,305
VF Filters	18	$352,800
Baculovirus Stock	780 L	$5,399,100
WFI	1,281,030 L	$3,727,798
Sodium Phosphate Monobasic	2387 kg	$372,408
Sodium Phosphate Dibasic	142 kg	$53,510
EDTA	143 kg	$19,249
Tergitol NP-9	94 L	$10,887
Glycerol	32,010 L	$88,918
NaCl	8,916 kg	$70,614
Ammonium Sulfate	19,837 kg	$43,733
NaOH	2,156 kg	$5,998
HCl	2,365 kg	$2,439
Ethanol	4,599 L	$18,349
Sodium Acetate	173 kg	$7,740
Vials	105,882,543	$39,176,541
Stoppers	105,882,543	$8,470,603
Seals	105,882,543	$14,823,556
HEPA Filters	3	$900

5.2.2. Fixed Operating Costs

Fixed operating costs include labor, supervision, overhead, administrative expenses, insurance, property tax, and maintenance. The plant requires 14 operators per shift across 4 shifts at a salary of $67,465, resulting in an annual labor cost of $3,778,040. Management and supervision are calculated as a factor of labor costs, totalling $944,510 annually. Direct salary overhead was estimated as a factor of both labor and management, and general administrative expenses as a factor of labor, management, and overhead costs. The standard multipliers used are outlined in Table 11-4. Property taxes and insurance costs were each estimated as 1% of the ISBL costs plus the OSBL costs annually, costing $16,737,589 annually per item. Maintenance costs estimated as a factor of ISBL costs (Table 11-3), totalling $2,041,169 per year.

The total fixed operating costs are $46,425,439 annually.

5.3. Revenue Estimation

Revenue is projected based on three market scenarios, broken down in Table 5.3-1.

Table 5.3-1 Revenue Modeling Scenarios

Scenario	Market Split	Price/Dose (USD)	Revenue (million USD/year)
Best-Case	100% Private Market	$7.81	$705.2
Mid-Case	50% Private, 50% Government	$5.56	$510.0
Worst-Case	100% Government Contracts	$3.50	$315.0

Costs per dose were determined by historical vaccine prices reported in Brazilian Reals (Crépey et al., 2020) and converted to U.S. dollars for comparative analysis and adjusted for inflation using consumer price indices to reflect 2025 values.

5.4. Total Cost of Production

The Cash Cost of Production (CCoP), including variable and fixed operating costs, totals $129,323,453. Selling & marketing costs are estimated to be five percent of the CCoP for a total of $6,466,173 annually. Costs for research and development (R&D) vary as a factor of revenue:

- Best-case: $105.8 million
- Mid-case: $76.5 million
- Worst-case: $47.3 million

The total cost of production is the sum of CCoP, selling and marketing, and R&D costs:

- Best-case: $241.6 million
- Mid-case: $212.3 million
- Worst-case: $183.0 million

5.5. Gross Profit and Income Tax

The gross profit after subtracting the total cost of operation from projected revenue is:

- Best-case: $463.6 million
- Mid-case $297.8 million
- Worst-case: $132.0 million

Assuming a straight-line depreciation of the total capital investment over seven years, and a corporate income tax of 15% in Brazil:

- The annual income after tax (Years 1-7):
  - Best-case: $396.0 million
  - Mid-case: $255.1 million
  - Worst-case: $114.1 million
- Post-depreciation income (After Year 7):
  - Best-case: $394.1 million
  - Mid-case: $253.1 million
  - Worst-case: $112.2 million

5.6. Financial Analysis

To assess the economic feasibility of the vaccine production facility, key financial metrics including Net Present Value (NPV), Return on Investment (ROI), Internal Rate of Return (IRR), and a Discounted Cash Flow Analysis (DCFA) were evaluated.

5.6.1. Net Present Value

The NPV quantifies the present value of projected future cash flows discounted at a rate, r, of 10%, reflecting the time value of money. It is calculated using Equation 5.6.1-1:

$$NPV = \sum \frac{C_t}{(1+r)^t} - C_0 \qquad \textbf{Equation 5.6.1-1}$$

Where C_t is the net cash inflow in year t, and C_0 is the initial capital investment. The rate chosen reflects the weighted average cost of capital (WACC), and the return rates usually required by pharmaceutical company investors are about 10-15%. The NPV for all 3 scenarios is positive, indicating profitability:

- Best-case NPV: $2.3 billion
- Mid-case NPV: $1.5 billion
- Worst-case NPV: $597.9 million

5.6.2. Return on Investment

The ROI compares net profit over a period of time with the capital investment. The formula is shown below:

$$ROI = \frac{Net\ Return - Initial\ Investment}{Initial\ Investment} \times 100 \qquad \textbf{Equation 5.6.2-1}$$

The return on investment for the 3 scenarios are show profitable investment:

- Best-case ROI: 3773%
- Mid-case ROI: 2423%
- Worst-case ROI: 1026%

5.6.3 Internal Rate of Return

The IRR represents the discount rate required for the NPV to become 0, and serves as a measurement of investment attractiveness.

$$0 = \sum \frac{C_t}{(1+IRR)^t} - C_0 \qquad \textbf{Equation 5.6.3-1}$$

For each scenario, the IRR exceeds typical discount rates of 10-15%, indicating the facility is financially viable:

- Best-case IRR: 393%
- Mid-case IRR: 258%
- Worst-case IRR: 113%

5.6.4 Discounted Cash Flow Analysis

A DCFA (Figure 5.6.4-1, Figure 5.6.4-2, and Figure 5.6.4-3) was conducted to evaluate the project's financial performance over 10 years of its expected operational lifetime. The cash flows considered include: revenue projections based on different pricing scenarios, operating costs including fixed and variable expenses, corporate income tax estimated at 15% in Brazil, depreciation calculated using the straight-line method, and total capital investment. This method also assumes no company growth, and thus is likely an underestimate.

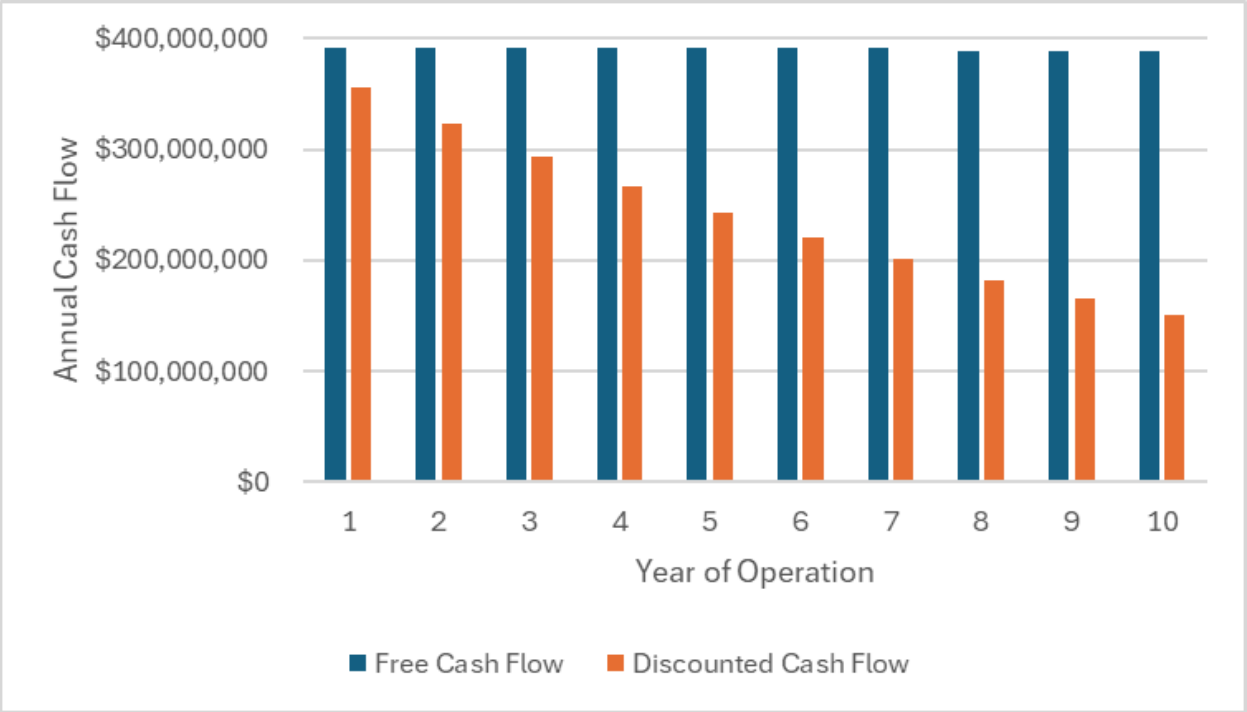


Figure 5.6.4-1. 10 Years of Discounted Cash Flows: Best-Case

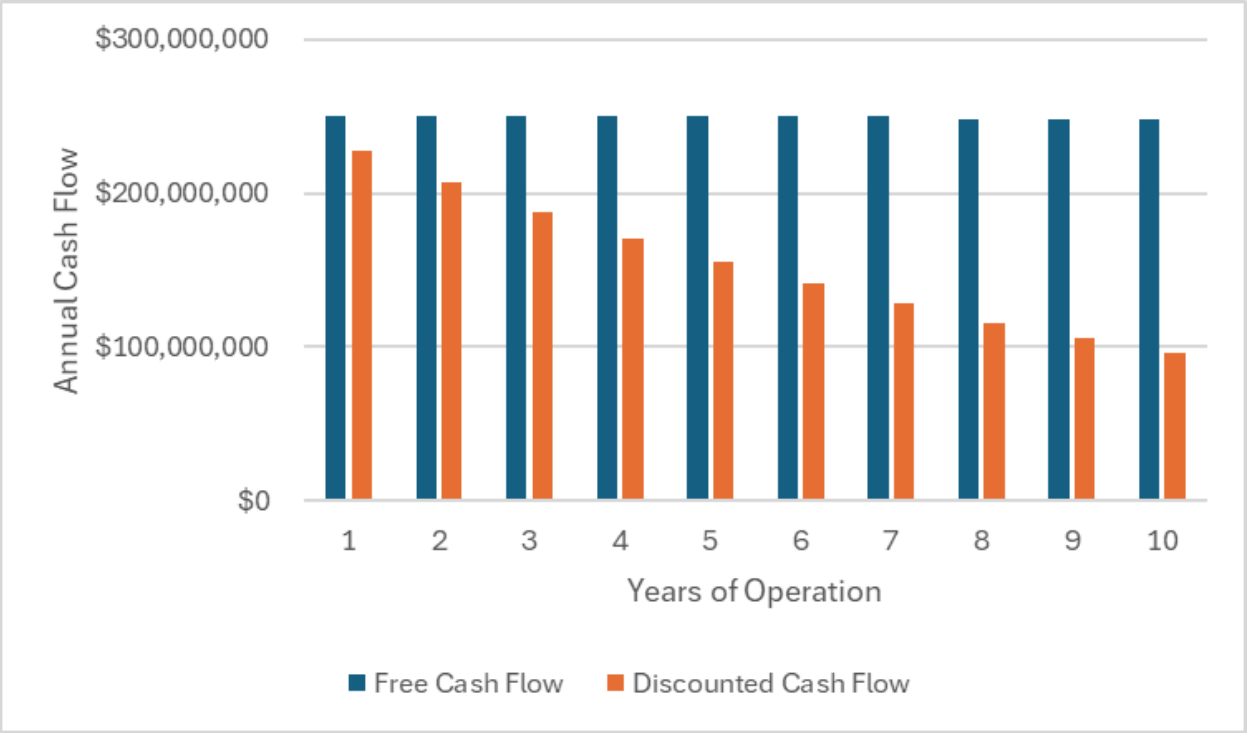


Figure 5.6.4-2. 10 Years of Discounted Cash Flows: Mid-Case

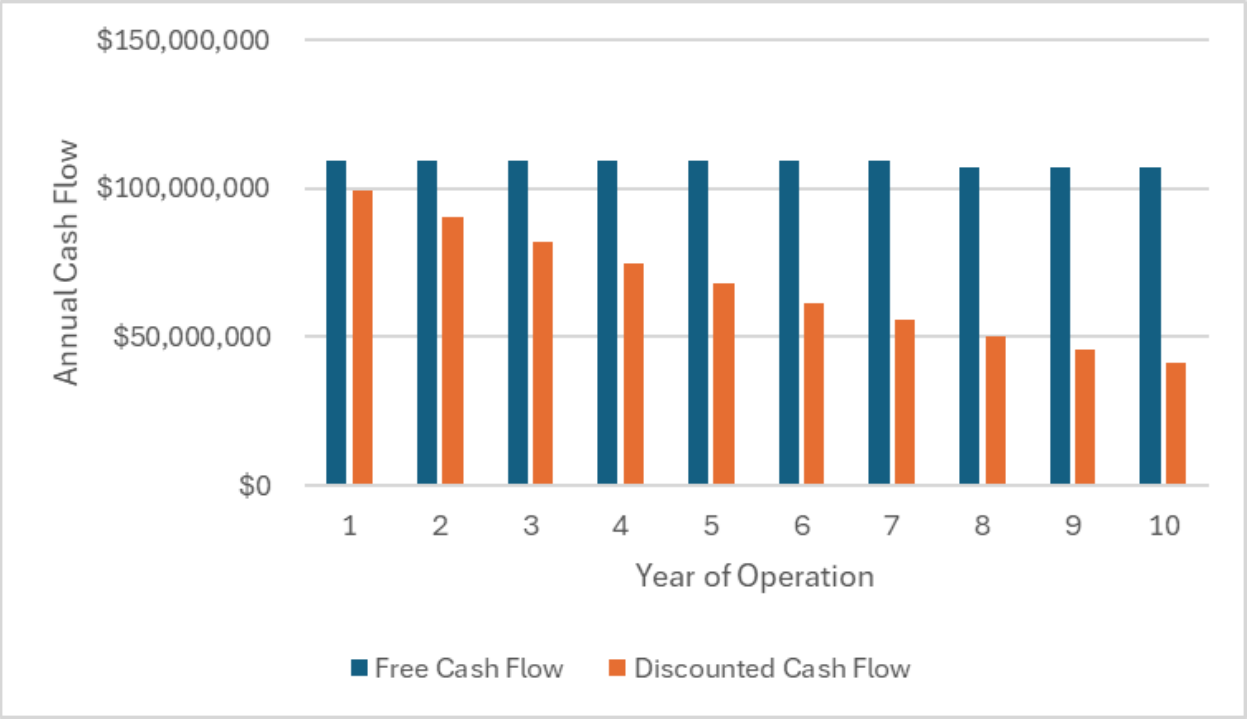


Figure 5.6.4-3. 10 Years of Discounted Cash Flows: Best-Case

6. Regulatory, Environmental, and Safety Concerns

In order to secure approval for distribution throughout Latin America, the Immunovida facility will strictly adhere to current Good Manufacturing Practices (cGMP) and to the guidelines set forth by the Brazilian regulatory agency ANVISA (Agência Nacional de Vigilância Sanitária). In the absence of available information from South American regulatory bodies, values from the United States will be substituted for the time being. In addition, efforts will be made to decrease the facility's environmental footprint without jeopardizing the quality and effectiveness of the final product. Given vaccine hesitancy and low vaccine uptake in the region, maintaining a relationship of trust with the consumer is of the utmost importance.

Another facet of maintaining a safe and reliable reputation is to prioritize employee welfare, be it through protective barriers or inherently safer design. These design choices are enumerated in the following sections.

6.1. Current Good Manufacturing Practices (cGMP)

The central tenet of current Good Manufacturing Practices is to deliver a product that is as safe, effective, and reliable as possible. The following strategies will be included in the operation of the Immunovida Flu Vaccine facility to best serve the Latin American market.

6.1.1. Data Integrity

The Immunovida facility will be operated on a completely paperless basis, with all batch records being stored electronically. This will reduce risk of batch records being physically destroyed, and will be cheaper to store in servers than in large warehouses. The basic ALCOA+ data integrity principles will be followed when recording batch information:

The data will always be:

1. Attributable
2. Legible
3. Contemporaneous
4. Original
5. Accurate
6. Complete
7. Consistent
8. Enduring
9. Available

6.1.2. Sampling & Batch Record Review

Margins of error are included in the manufacturing process, such that the final volume of vaccine produced allows for regular sampling. Despite there being extensive precautions taken to purify the final product and ensure the aseptic status of the facility, rigorous testing will occur to ensure that regulatory guidelines regarding the presence of bacterial contamination, viral contamination, endotoxins, and other contaminants are met.

Following the completion of one batch, the filled vials will be held in storage until the completion of batch record review. Only after all samples and batch records have been deemed satisfactory will the batch be approved for dispatch to a labeling and packaging facility.

6.2. Chemical Compatibility

The chemical compatibility chart summarized in Figure 6.2-1 below shows the general National Fire Protection Association (NFPA) hazards associated with every chemical used in the process. Generally, all the chemicals are very low risk, with the highest concerns being the flammability of ethanol and the health hazard of HCl, NaOH, and Tergitol NP-9. To prevent and mitigate any incidents occurring with these substances, the sanitization buffer and ethanol tanks will be isolated away from the rest of the process, sprinklers will be installed in the plant, and employees will be required to wear proper PPE.

Y : Compatible
N : Incompatible
C : Caution
SR: Self-Reactive
*: Changed by user

NFPA Health	NFPA Flammability	NFPA Instability	NFPA Special	Flu Vaccine Compatibility Chart	AMMONIUM SULFATE	EDTA	ETHANOL	GLYCERINE	HYDROCHLORIC ACID, SOLUTION	Sodium Acetate (Anhydrous)	SODIUM CHLORIDE	SODIUM HYDROXIDE SOLUTION	Sodium Phosphate (Monobasic)	SODIUM PHOSPHATE, DIBASIC	Tergitol NP-9	WATER	
				AMMONIUM SULFATE													
				EDTA	C												
2	3	0		ETHANOL	Y	N											
1	1	0		GLYCERINE	Y	N	Y										
3	0	1		HYDROCHLORIC ACID, SOLUTION	C	N	C	C									
0	1	1		Sodium Acetate (Anhydrous)	Y	Y	Y	Y	C								
				SODIUM CHLORIDE	Y	C	Y	Y	N	Y							
3	0	1		SODIUM HYDROXIDE SOLUTION	N	N	N	N	N	Y	Y						
0	0	0		Sodium Phosphate (Monobasic)	Y	C	Y	Y	N	Y	C	N					
				SODIUM PHOSPHATE, DIBASIC	N	C	Y	Y	N	Y	C	N	C				
3	1	0		Tergitol NP-9	N	Y	Y	Y	N	Y	Y	Y	Y	Y			
				WATER	C	C	Y	Y	C	Y	Y	C	C	N	Y		

Figure 6.2-1. Chemical Compatibility Chart

6.3. Waste Disposal

The Sf9 and baculovirus expression system platform present a uniquely low biohazard. Both the cells and the virus are rated biosafety level one (BSL-1) materials (American Type Culture Collection, 2024; USC Environmental Health and Safety, 2023), meaning they "are not known to consistently cause disease in healthy adults and present minimal potential hazard to laboratorians and the environment" (Centers for Disease Control, 2025). As a result, media can be autoclaved and sent to regular liquid waste management facilities. However, the sheer volume of media being expensed by the facility makes it unrealistic to autoclave this biohazardous material on site. Therefore, all cell media, excess baculovirus, and solid cell waste will be disposed of as hazardous waste. It is feasible to autoclave the cell pellets on site, but the relatively small amount of solid cell waste makes it a trivial addition to shipments of other biohazardous waste.

All buffers are disposed of as hazardous liquid waste because of their potential to contain residual biohazards, chromatography resin, viral contaminants, and other contaminants. The sanitization buffer in particular is neutralized with hydrochloric acid to produce a 1.5M NaCl aqueous solution before disposal. All single-use solid waste is also disposed of as hazardous waste for the same reason. It is feasible to autoclave the single-use solid waste as well, but it is far more convenient to have it processed as hazardous waste professionals given the relatively small amount of solid waste on site.

Lastly, all chromatography resins are disposed of at the end of the season. These resins need to be cured under UV light in order to be disposed of as household waste. Else, they must be discarded as hazardous waste.

For simplicity, waste management costs were estimated with all waste streams classified as hazardous waste and disposed of at a rate of $500 per ton (Table 6.3-1). This estimation was derived from a range of $200-$2000 per ton of hazardous waste disposal in Turton et al. (2012). Given that the materials in question are low-level biohazards and are being processed in bulk, an estimate on the lower end of that range was selected. Turton et al. note that “escalation of these costs should be done with extreme caution,” remarking that the cost of managing biohazard waste has risen significantly in recent years (2012). However, the bulk disposal of the materials from this facility in combination with significant technological advances in the years since 2012 prompted the assumption that the estimates provided by Turton are still applicable in 2025. No adjustments were made for inflation, as it was assumed that technological advancements were assumed to decrease the cost of waste disposal by a value greater than the increase due to inflation. The density of all liquids was approximated as that of water. The density of the chromatography resin was assumed to be 0.76 kilograms per cubic meter. In the absence of specifications for the mass of single use materials, approximations were made.

Table 6.3-1 Estimated Waste Disposal Costs

Waste Category	Waste Type	Quantity per Year	Units	Cost (USD)
Solid Cell Waste	Solid	4445	kg	$2,222
Neutralized Caustic	Liquid	120013	L	$60,007
Waste Buffer A	Liquid	488996	L	$244,498
Waster Buffer B	Liquid	64800	L	$32,400
Waste Buffer C	Liquid	86400	L	$43,200
Waste Buffer D	Liquid	300240	L	$150,120
Waste Buffer E	Liquid	30780	L	$15,390
Waste Storage Buffers	Liquid	22995	L	$11,498
Waste Formulation Buffer	Liquid	3082	L	$1,541

Table 6.3-1 Estimated Waste Disposal Costs

Waste Category	Waste Type	Quantity per Year	Units	Cost (USD)
Waste Media	Liquid	1440000	L	$720,000
Waste WFI	Liquid	64260	L	$32,130
Single Use Materials	Solid	11036	kg	$5,518
Chromatography Resins	Solid	657	L	$250

6.3. Equipment Hazards and PPE

The biggest risk posed to personnel is the caustic and neutralizing acids held on site. Employees handling acids and bases should be sure to review the appropriate SOPs and handle these materials with an abundance of caution. An in depth analysis of a possible caustic release incident is detailed in Section 6.5. Notably, the risk of injury as a result of caustic release would be much higher if single-use equipment were not in use, as much more caustic would be circulating around the facility. However, single use equipment comes with its own risks. Bioreactor and tank linings must be installed with extreme caution to ensure preservation of the aseptic space. In addition, the replacement of single use tubing poses some risk. Non-permanent tubing faces much higher odds of bursting or otherwise disconnecting from ports as a result of pressure jumps. Tubing may not be completely dry during replacement periods, so employees should take care to wear the proper PPE to prevent being splashed with residual material. All piping should be properly stowed in overhead piping racks and off of the floor to prevent trips and falls.

The large bioreactors and holding tanks pose an inherent equipment hazard. As such, precautions must be taken to limit risk of employees falling into the large tanks. The SOPs detailing the changing of tank liners must be written with extreme care to ensure that no

employee is ever at risk of endangering themselves. All work done around the top of the tanks should be performed with harnesses and at least two operators.

Some safety hazards can be mitigated with the use of proper PPE. Given that the Sf9 cells and baculovirus represent a BSL-1 hazard, a lab-coat or equivalent, eye protection, and gloves must be worn at all times. This is already accounted for by the Grade D and higher cleanroom protocols enacted in this facility.

6.4. Inherently Safer Design

A variety of inherently safer design decisions were made to limit risk within the Immunovida facility. For example, the decision to utilize as many single-use elements as possible will limit employees' exposure to hazardous caustic and the corresponding neutralizing acid. Furthermore, the use of the Sf9 and baculovirus expression system reduces the overall biosafety level of the facility. The facility is rated BSL-1, whereas the use of mammalian cells or viruses capable of infecting humans would increase the level of inherent risk associated with the operation.

The temperature at which fermentation occurs at, although not necessarily a design choice, does make the facility inherently safer. Rather than the typical 37°C used for a variety of other cell cultures, Sf9 fermentation takes place at 27°C. As a result, the conditions are inhospitable to a variety of bacterial contaminants. It is also easier for process piping to withstand these close-to-ambient temperatures, and the fermentation requires no warming jackets or heat exchangers to maintain a steady temperature. The elimination of such equipment removes all possibility of heating related malfunctions.

6.5. Most Credible Event

The most credible event for this facility was determined to be due to a pump seal failure on the hydrogen chloride tank used to neutralize waste. Because hydrogen chloride was the only volatile substance out of all the materials that had an NFPA hazard of three, this substance was determined to be the most hazardous in the system. The softwares MARPLOT and ALOHA were used to model the release scenario of 31% hydrogen chloride solution. The scenario was modeled 50 miles south of the current Instituto Butantan facility due to the location being flat and isolated from civilians.

Results from this simulation are depicted below in Figure 6.5-1., showing a toxic threat zone of up to 1,000 yards. Because of these results, the facility should remain isolated from populous cities and employees should make sure to wear gloves, protective clothing, and face protection when neutralizing waste. Additionally, the compatibility chart in Figure 6.2-1 shows that hydrogen chloride should not come in contact with a majority of the substances in the facility, meaning that this tank should be placed away from the other buffer tanks.

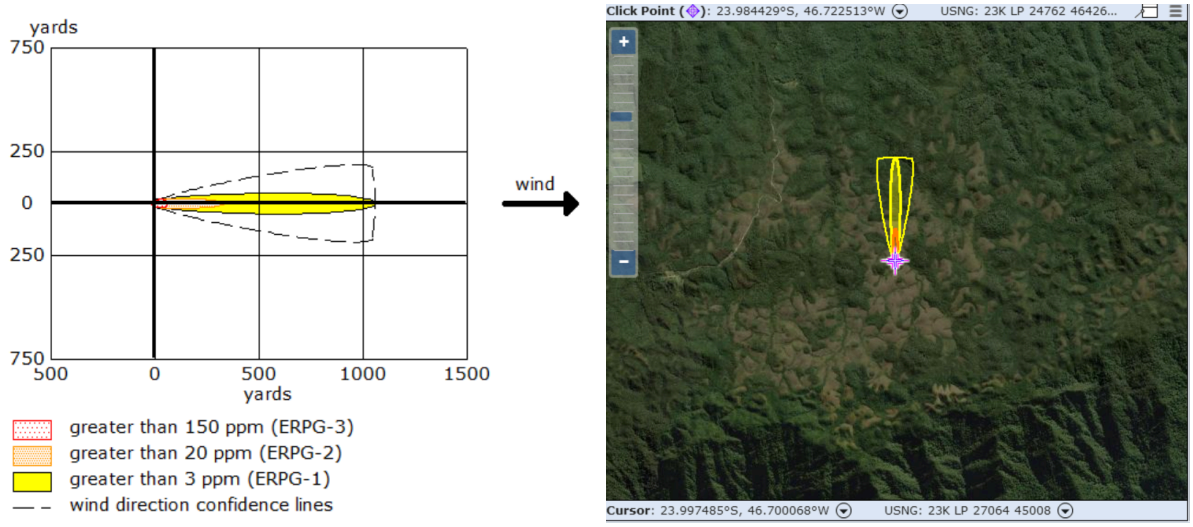


Figure 6.5-1. Toxic Threat Zone of Most Credible Release Scenario

7. Societal Impact

The societal and ethical considerations of this project involve the establishment of a manufacturing facility within an existing community, which will impact the local residents by integrating them into the culture and values promoted by the facility. Local residents will be employed by the plant, creating valuable job opportunities and fostering economic growth in the community. We are committed to adhering to Brazil's established customs and workplace regulations to ensure a positive working environment. This includes offering fair wages, maintaining high standards for working conditions, and providing thorough training on our materials, processes, and product. Safety, ethical, and diversity training will also be mandatory for all employees before they begin their work. Additionally, the presence of this facility may help combat any vaccine hesitancy and circulating misinformation in the area by shifting public perception through direct engagement and education.

A challenge also exists in determining a pricing strategy that ensures the vaccine is both affordable and accessible while balancing financial stability. This strategy might involve prioritizing public vaccination programs over private sales, dedicating a larger share of doses to initiatives led by organizations like the Pan American Health Organization's (PAHO) Technical Advisory Group on Vaccine-Preventable Diseases (Gentile et al., 2019). Ensuring effective tracking of vaccine distribution will be essential in assessing whether the product is reaching all populations, particularly those from underserved and economically disadvantaged areas.

8. Conclusions and Recommendations

Given the profitability and positive societal impact of increased influenza vaccine production for the global south, our recommendation is to continue with plans to construct this facility. Increasing isolationism in global politics makes it imperative that South American countries like Brazil are capable of meeting domestic vaccine demand without depending on foreign producers.

Prior to production, we recommend further research in the following areas. Primarily, a greater understanding of Sf9 cell kinetics and product formation kinetics would ensure maximum efficiency in the facility. For the purpose of this investigation, recipe-style kinetics were used in the place of more descriptive Monod kinetics due to a lack of information regarding rate of product formation. Furthermore, broad assumptions were made regarding Sf9 oxygen consumption and the sparging requirement to meet it. We are confident in our recommendation of a specialized microsparger, but future groups should consider designing this sparger for themselves or seeking to further understand the technology presumably used by Buckland et al. (2014).

Furthermore, more consideration should be given to baculovirus sourcing and waste disposal methods. The development of an in-house baculovirus expansion unit was not within the scope of this investigation, but would assist in building the independence of the facility.

Future teams should continue to consider single-use equipment in the place of hazardous CIP and SIP chemicals. Additionally, processing hazardous waste in-house could lower waste disposal costs. Notably, future groups should consider neutralizing their caustic with something other than hydrochloric acid to reduce risk of dangerous release incidents.

Lastly, future teams should be open to the possibility of different formulations of the recombinant influenza vaccine. For example, trivalent vaccines or vaccines containing specialized adjuvants. As more information becomes available, these avenues could provide the Immunovida facility with more opportunities to make a positive impact on the community.

In conclusion, a recombinant quadrivalent influenza vaccine for Latin America presents an opportunity to promote public health and stimulate the local economy. The BEVS platform is flexible and reliable, allowing for short reaction times to new strains and low biohazard risk on site. It is our recommendation that this project proceeds immediately.

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11. Appendix

Table 11-1 Chromatography Buffers

Buffer Name	Formulation	pH	Source
Buffer A	20 mM Sodium Phosphate 1.0 mM EDTA 0.01 % Tergitol-NP9 5% Glycerol	5.89	(Wang et al., 2006)
Buffer B	20 mM Sodium Phosphate 0.03 % Tergitol 5% Glycerol	7.02	(Wang et al., 2006)
Buffer C	20 mM Sodium Phosphate 150 mM NaCl 0.03% Tergitol 5% Glycerol	7.02	(Wang et al., 2006)
Buffer D	20 mM Sodium Phosphate 0.5 M Ammonium Sulfate	7.00	(Li et al., 2018)
Buffer E	20 mM Sodium Phosphate, 0 M Ammonium Sulfate	7.00	(Li et al., 2018)
Sanitization	1 M NaCl 0.5 M NaOH	14.00	Cytiva Operating Manual

Table 11-2 Parameters for Equation 5.1

Equipment	a	b	n	S
Stainless Steel, Jacketed, Agitated Bioreactor	61,500	32,500	0.8	0.5-100 m^3
Cone Roof Tanks	5,800	1,600	0.7	100-10,000 m^3
Propeller Mixer	17,000	1,130	1.05	5-75 kW
Blower Compressor	4,450	57	0.8	200-5,000 m^3/h

Table 11-3 Factors Applied to ISBL Costs

Outside Battery Limits	Design and Engineering	Contingency	Maintenance
0.4	0.3	0.1	0.05

Table 11-4 Factors Applied to Estimate Fixed Cost of Production

Management and Supervision	Direct Salary Overhead	General and Administrative
0.25	0.4	0.65