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Production of Trivalent Influenza Vaccines Disposable Technology and Insect Cell Lines

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Dr. John Schlup

Abstract:

The overall production for the entire process is 22 million doses per year. This production capacity is approximately the same as a medium sized facility. Using a baculovirus insect cell expression system can reduce the production time of influenza vaccines from 6 months to 4 months. The other cell line considered, Chinese Hamster Ovaries, was determined to be unfavorable due to safety concerns. It was determined that disposable technology would be ideal for the process, as it allows production scale up with minimal capital investment. The disposable technology employed in the facility was found to be more economically viable than conventional multi-use technology. The facility still requires the use of some multi-use components, such as chromatography and diafiltration, but single use technology is employed where possible. Examining the project, a lifespan of 23 years was determined to be appropriate for demonstrating the economic feasibility of the project. It was found that the minimum IRR at current prices was 33.36%. With a 12% discrete interest rate, the net present value of the facility is $278 million, for a total capital investment of $149 million. At this rate, it was also determined that the payback period was 2.83 years with a return on investment of 34.42%. From this data, it was concluded that the project should proceed as planned.

Keywords: Trivalent, Influenza, Vaccine, Facility, Disposable, Insect

Memorandum

To: Plant Manager Calvin Langford
From: Senior Project Engineer Andrew W. Woolley
Date: March 28, 2014
Subject: Evaluation of a Production Process for Trivalent Influenza Vaccines

The following report contains information regarding the evaluation of a project for the production of trivalent influenza vaccines. In order to replace the older facility, it was determined that a production of 22 million doses per year was needed. For the facility, two cell lines were considered, and it was determined that a baculovirus insect cell expression system would best suit the needs for the new plant.

Initially, it was decided that a disposable technology plant design would be utilized. The economic evaluation confirmed this decision. A time scale evaluation determined that the final design could have the entire production line out to market in just short of 4 months, 2 months earlier than the older egg-based influenza facility. A final economic analysis determined that the process is economically feasible in the current market, and the project should go through to the next design phase.

Assistance was provided by the teams at F.Z. Vaccines, our sister company. Information provided by the teams allowed for the design of the upstream process using the kinetic data from the literature they provided on cellular growth and material consumption. Specific equipment costs were provided by suppliers upon request, post design stage. The team consisted of myself.

Regards,

Andrew W. Woolley

Production of Trivalent Influenza Vaccines: Disposable Technology and Insect Cell Lines

Source: PM Group. http://www.pmgroup-global.com/Expertise/focus-on---/Focus-On-Pages/Vaccines-Asia-%281%29.aspx

Submitted To:

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Plant Manager
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Submitted By:

Andrew W. Woolley
Senior Project Engineering
March 28, 2014

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Abstract

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The disposable technology employed in the facility was found to be more economically viable than conventional multi-use technology. The facility still requires the use of some multi-use components, such as chromatography and diafiltration, but single use technology is employed where possible. Examining the project, a lifespan of 23 years was determined to be appropriate for demonstrating the economic feasibility of the project. It was found that the minimum IRR at current prices was 33.36%. With a 12% discrete interest rate, the net present value of the facility is $278 million, for a total capital investment of $149 million. At this rate, it was also determined that the payback period was 2.83 years with a return on investment of 34.42%. From this data, it was concluded that the project should proceed as planned.

1.0 Introduction

Influenza is a current world health issue. Due to the highly infectious nature of the virus, it can easily spread from individual to individual. A global pandemic due to the influenza virus is foreseeable in the future. Thus, it is necessary to develop high yield quick response systems for inoculating the public against the spread of the influenza virus.

Vaccination is the current method of preventing infection within in the public. But in the United States, approximately 134.5 million dose were distributed in the 2013/2014 season, which is only a fraction of the population [1]. After brief market analysis using these data, and then combing them with other market information, an interesting trend was noticed. It was found that influenza consumption increased from the 2000/2001 season, peaked in the 2010/2011 pandemic flu season, and then has steadily declined to its current state in the 2013/2014 season [1, 2, 3]. The data indicate that the demand for influenza vaccines, while large, is not inherently stable, and can quickly go up or down from year to year.

1.1 The Two Technologies

This means that there is a need for a flexible manufacturing process when it comes to influenza vaccine production. Current conventional technology is not inherently capable of performing this. In order to meet fixed production costs related to capital demands, conventional technology has to produce a minimum amount to remain economically feasible. This forces production plants to stray from the optimum the production point, leading to higher costs per unit produced in the process.

When looking at single use disposable technology, this issue disappears. While there are some capital requirements, the fixed costs due to capital are significantly reduced. What is more, batches are produced in smaller quantities, which allows a more refined approach to production. Instead of having to scale a batch to meet the demand, production simply has to scale the number of batches to the demand. The inherent disadvantage to the process is that variable costs go up. Each batch requires higher operational costs due to the fact that disposable bags, filters, and other items have to be purchased. This leads to the conclusion that there is a certain point at which a disposable technology facility becomes less economically viable than a conventional one when it comes to larger scale production.

1.2 Inadequacy of Egg-Based Production

There is another inherent issue with current influenza vaccine production. Most vaccines are produced using an egg-based production method. While this method has been shown to be effective, there are some issues with this process. The first is that egg-production methods require the preorder and accumulation of millions of chicken eggs. This requires a lead time of up to a year for each batch production year [4]. With a year of lead time, there is little room for mistakes during the production process. If a distinct batch goes bad, the chances are that multiple other batches are bad, and that recovery from a mistake is impossible.

The chance of an ineffective batch is, in fact, very high. Influenza is known for its mutating abilities, and could potentially change before the production process is completed. The result is

a vaccine that is ineffective for that year of production, and either cannot be sold, or has to be sold at a reduced price due to its reduced effectiveness, if at all possible.

The other inherent issue with egg based vaccines is the potential for contamination during avian influenza seasons [5]. This can lead to reduction in influenza vaccine stocks during avian influenza pandemics. The result is an inadequate amount of vaccine due to the feedstock used for production.

The last reason for the need to replace egg-based vaccine approaches is individuals with allergies. Egg-based processes can leave residual ovalbumin in the vaccine, which can cause an allergic response in individuals who receive the vaccine. Thus, this drawback reduces the market for the vaccine.

These reasons lead to the conclusion that a faster paced, more robust, in terms of feedstock and product, process is necessary. It has been proposed that cells can be grown and used to produce vaccines. The two current cell lines are Chinese Hamster Ovaries (CHO) and baculovirus insect cell systems. Both processes have inherent advantages and disadvantages when it comes to processing.

1.3 CHO Cell Lines in Brief

CHO is a mammalian cell line. It's abilities to produce are well established, as over 70% of all recombinant proteins are made in CHO cell lines. They allow for volumetric scalability in bio-reactors and can grow to titers between 1-5 g/L [6]. The disadvantage of these lines is the need for live influenza virus strains in the process [7]. Due to the fact that there are live influenza strains used in the process, better security measures are needed to protect employees from infection. In fact, some information indicates the need of a Bio-Safety Level 3 (BSL-3) facility for the production of certain avian influenza strains [5]. With CHO cells, there are more issues that will be discussed further in section 3.0.

1.4 Baculovirus Insect Cell Systems in Brief

The other lines considered were the baculovirus insect cell expression systems. Insect cell lines typically used for large scale production are the Sf9 or Sf21 variety from Invitrogen. These cells can be easily grown in serum free media and have been shown to produce product that is highly effective in humans [5]. Furthermore, two processes have been developed by both Novavax [7] and Protein Sciences [8], with Protein Science's FDA approved Flublok line being based upon this method of production. This step shows that the ability to produce recombinant influenza protein vaccines with insect cell lines is possible.

Also, baculoviruses are only infectious to arthropods [9]. This means that the production process, while still BSL-2, is inherently safer for both employees and individuals receiving the vaccine. Novavax [7] and Protein Sciences [8] both state that their processes do not contain inactivation steps, due to this fact. Thus, processes using this technology would have reduced materials and capital costs because of the lack of an inactivation step.

Baculovirus insect cell expression systems are temperature and pH sensitive, though [10]. This means that carful in process monitoring is required to use these systems. This also means that

the reactors used to produce these lines will be inherently costly, due to the controls needed for the process. The insect cell lines also grow at a pH of 6.2 to 6.4, while the desired product protein, hemagglutinin, can be denatured at a pH of 5.5 [11]. While a buffer solution can prevent the pH from becoming too low, it is possible that a batch could be ruined due to a low pH, leading to higher production costs if too many batches are lost.

1.5 Looking towards the Design

It is clear that the current methods used for producing influenza vaccines are inadequate. Supplies can easily be disrupted by disease, while long lead times can make the process sensitive to changes in the virus. What is more, the market is limited due to allergens present in product. The above information shows that there a better possibilities when it comes to the production of trivalent influenza vaccines in terms of both technology and media/feedstocks. This information leads to the conclusion that a better design can be developed. The development of said design is the scope of this report, and further explored in the following pages.

2.0 Process Flow Diagrams and Material Balances

For process modelling purposes, a design was run in SuperPro Designer v8.5. Due to the limitations of SuperPro (only allowing 25 units per sheet) and the complexity of the design, the upstream and downstream processes were separated into two individual sheets, and their mass balances performed separately.

2.1 Upstream Processing

An overall mass balance of a single batch for the upstream process is provided in Table 1, while the process layout is provided in Figure 1. Individual units were first balanced in order to determine if the discrepancies between the inlet and outlet flows were negligible (< 1% error). None of the individual units were found to have discrepancies during this portion of the mass balance process, though, each reactor did have a small discrepancy.

Table 1: Upstream Mass Balance

Overall Mass Balance Upstream		
Component	**Input (g/batch)**	**Output (g/batch)**
Ammonium	0.00	0.84
Carb. Dioxide	0.00	2.05
Cell Biomass	0.00	554
Glucose	5984	5975
Haemagglutinin	0.00	25.17
L-Glutamine	997	990
Lactate	0.00	4.30
Media	23736	23166
NaHCO3	349.06	349.06
Nitrogen	293780	293780
Oxygen	89186	89467
WFI	966245	966245
Total	1380277	1380559
Discrepancy (g/batch)		-282.49

When performing the calculations for the upstream process mass balance, a discrepancy was found between the amounts of mass flowing in and out of the process. An investigation found that large amounts of nitrogen were being consumed (according to SuperPro) during the process. This discrepancy was rectified by hand in an MS Excel mass balance table in the overall mass balance section. It was assumed that nitrogen is an inert in the process. By fixing this, the discrepancy was reduced from -1200g to -282g, which was evaluated to be negligible when looking at the total mass flow of the process (< 1% error). Thus, the mass balance was found to come to near complete unity.

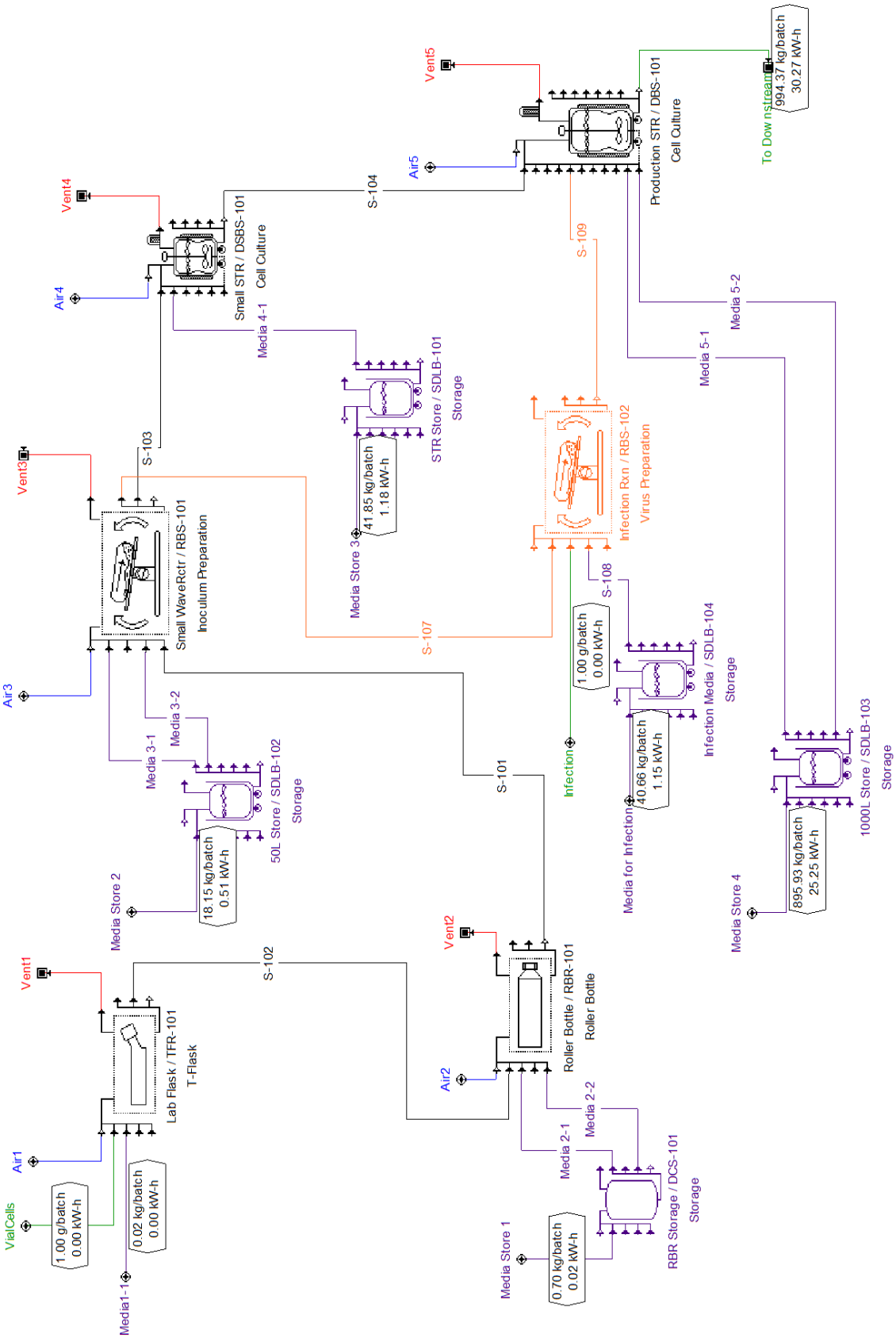

Figure 1: Upstream Process Flow Diagram with Mass and Energy Flows

Coloring is provided in order to aid the reader in following the process. When looking at Figure 1, it should be noted that the infection bioreactor is sized as a 200L bioreactor. This is a potential design area of improvement, as a using a 100L working volume bioreactor as a substitute for a 50L working volume wave bioreactor can make the process somewhat less efficient when it comes to storage. Modifying a wave bioreactor for a smaller volume should be considered for the future.

2.2 Downstream Processing

The mass balance for the downstream process came to unity without any issues during the evaluation. Individual mass balances were performed over each unit, and each unit was found to have negligible errors. When the total mass balance was performed, again, negligible discrepancies were found in the process. The total mass balance is summarized in Table 2.

It should be noted that there are some components of the mass balance table that are zero in and out. These components were originally part of the design, but later eliminated when deemed unnecessary. It was also assumed that the process was air tight for simplification of the calculations. This assumption is not 100% accurate, as parts of the process can come into contact with air. It was assumed that Good Manufacturing Practices would be performed and contact with potential contaminants, such as air, would be eliminated.

Table 2: Downstream Mass Balance

Overall Mass Balance Downstream		
Component	**Input (g/batch)**	**Output (g/batch)**
Ammonium	0	0
Carb. Dioxide	0	0
Cell Biomass	554	554
Glucose	5958	5958
Haemagglutinin	25.2	25.2
HCl	2.84	2.84
Inulin	8500	8500
Isopropanol	2637	2637
KCl	0	0
KH2PO4	0	0
L-Glutamine	987	987
Lactate	4.30	4.30
Media	23096	23096
Na2HPO4	737	737
NaH2PO4	182	182
NaHCO3	348	348
Nitrogen	0	0
Oxygen	0	0
Sodium Chloride	9707	9707
Sodium Hydroxid	435	435
Water	0	0
WFI	1670098	1670098
Total	1723272	1723272
Discrepancy (g/batch)		-0.027

Figure 2 shows a general process overview of the downstream process. Multiple upstream processes can utilize a single downstream process, which is why there is a separation between the process flow diagrams.

It should be noted that some streams do not contain mass flow rates, as they are simple cleaning steps. Other streams are not included due to the potential amount of clutter that adding a label to these streams would create. Lastly, some streams are presented in kilograms, while others are in grams, which could potentially confuse the reader if unnoticed.

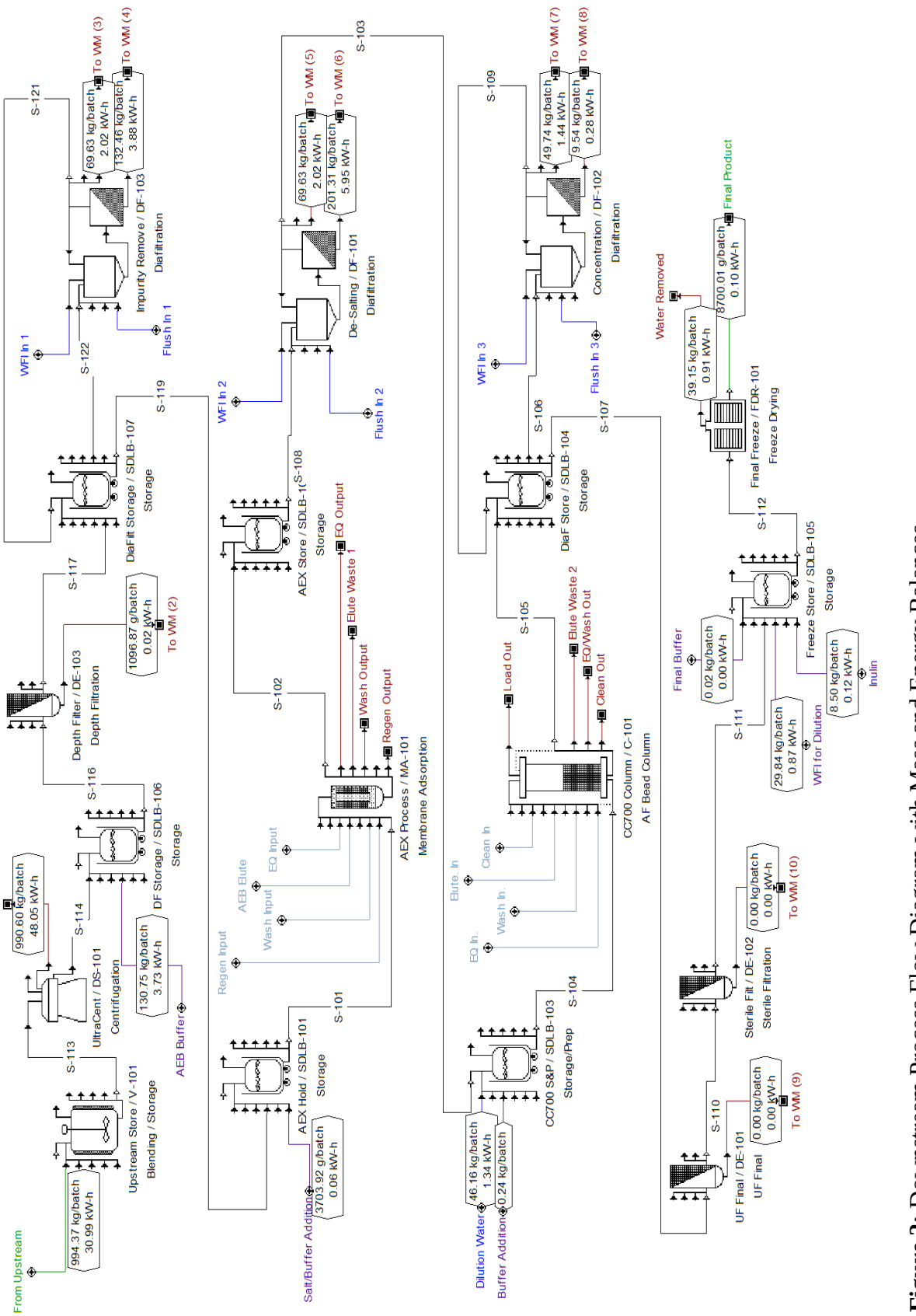

Figure 2: Downstream Process Flow Diagram with Mass and Energy Balances

3.0 Process Description

Both the upstream and downstream processes had multiple considerations and evaluations that had to be performed. This description follows the process in the order of the stream flows.

3.1 Upstream Processing

For the upstream processing side, there were three primary considerations that had to be examined. The first consideration was the cell line that was going to be used. As stated in the introduction, there were a number of issues regarding both cell lines, but ultimately insect cells were chosen for use. The second consideration was to use disposable or conventional technology for the bioreactor processes. Initial research indicated that disposable technology was more favorable than conventional technology. After designing the process, detailed economic evaluations were performed in order to compare both technologies, and it was found that this decision was justified. The final consideration was whether to manufacture or purchase the media for cellular production. Manufacturing media proved to be more difficult, as most formulations are confidential, and cell lines show preferences when it comes to processing.

3.1.1 Cell Line Decisions

The two cell lines considered were CHO and insect cell systems. The first cell line considered was CHO. CHO presented a viable solution, as over 70% of recombinant proteins are produced using CHO cellular expression systems. They can be grown in high concentrations and provide easier volumetric scalability when it comes to batch modelling [6]. There are a number of inherent disadvantages, though, when it comes to processing with these types of cells.

Due to the fact that CHO are mammalian cells, they require live influenza virus in order to infect [7]. While this makes relating the vaccine to humans easier, it means that more stringent safety measures are required when it comes to processing these types of cell lines. In fact, some evaluations recommend using BSL-3 facilities when it comes to using these cell lines and avian influenza viruses, due to the danger presented to employees while processing [5]. Higher level facilities require more stringent regulations and methods of control when it comes to processing, increasing costs along with inherent danger.

A second similar issue arises in CHO cells as well. Due to their relatability to humans, other human viruses can more easily contaminate the cellular stock [12]. Add in the fact that CHO cells are highly susceptible to lysing when subjected to shear forces (such as mixing) [13], and the overall process using this cell line becomes more costly due to more evaluations and testing, and less robust when it comes to process designs.

Insect cell lines showed immediate promise. Experimental evaluations showed that vaccines produced from insect cells were effective in humans. The added fact that insect cells could easily be put into chemically defined serum free media without adaptation made them favorable for the process [5]. Further examination found that both Novavax [7] and Protein Sciences [8] had developed processes for influenza vaccine production using insect cell lines. Insect cell lines, such as Invitrogen's Sf9, which can be used in large scale processing, could also be grown in suspension at 27°C [10]. This meant that energy inputs for heating the cells would be minimal, as this temperature is barely above room temperature.

Another advantage to insect cell lines is the fact that baculoviruses, the viruses used to infect insect cell lines, are only infectious to arthropods [9]. Two sources, Novavax [7] and Protein Sciences [8], confirm this. Both companies stated that a downstream inactivation step was unnecessary when it came to processing. In many cases, hazardous chemicals such as formalin, are used for the inactivation step downstream. Eliminating this hazardous step completely not only reduces costs, but also follows some of the guidelines set by the 12 Principles of Green Engineering [14]. Thus, a better process could be designed using insect cell lines.

Some disadvantages for insect cell lines became clear, though. While lines, such as Sf9, are robust when it comes to shear forces, factors such as pH and temperature can greatly inhibit growth [10]. What is more, insect cells grow at a pH around 6.2 to 6.4, while hemagglutinin, the target product, can be denatured at a pH of 5.5 [11]. This could potentially lead to a higher amount of batches lost during processing, due to denaturing of the product.

Based on the information stated above, it was decided to use insect cell lines, and more specifically, Sf9 insect cell lines. Sf9 insect cells show robust behavior during processing [10], produce minimal waste products that inferred with growth [15], and have ample research on kinetics data and behavior in process type environments [10, 15, 16, 17]. Some sources had quantified production quantities of hemagglutinin produced based on total amount of cells [17], which reduced the difficulty of designing the process. Lastly, there is already an insect cell line based influenza vaccine entering the market (Flublok), showing that the process can be developed successfully [18].

3.1.2 Technological Decisions: Disposable or Conventional

There were two technologies considered: Disposable and conventional. It was determined early on in the process that disposable technology would be the best possible method when it came to the bioreactor process train.

Current conventional technology uses Steam-in-Place (SIP) and Clean-in-Place (CIP) processes after each run. This requires large amounts of capital investments to be made in the process. It was estimated that the cost of a conventional style facility would be around $600 million. A similar sized disposable facility was found to have a capital investment of only $40 million, 12x less than the conventional design [19]. Other economic comparisons based on conventional technology found the net present value (NPV) of a conventional facility was higher, but factoring in time till production increased the NPV of disposable technology [20].

Looking further into the technology, it was found that every upstream process had a disposable version. While this increased operational costs of the facilities, this trade off was deemed effective in terms of long run economics. In the long run, initial investments have high values while revenue returns diminish over time due to inflation, interest, and other external factors. Thus, a smaller initial investment with slightly larger operational costs in the future was deemed more economically viable. Both processes, though, were evaluated in order to ensure this assumption was correct and is discussed further in section 12.0 of this report.

Another factor that was considered was sterility of the process. As stated earlier, conventional technologies use SIP and CIP methods, while disposable technology is simply one use and done.

The bags used in disposable processing are certified as sterile when delivered, thus reducing the need for validation. On the other hand, conventional technology requires extensive validation, while still having an inherent risk of cross contamination and carryover between batches [21]. This could potentially lead to more batches lost during production, due to contamination, and thus, increasing the costs of the process or potentially limiting the process production altogether.

There was one final consideration which was a Contract Manufacturer (CMO). This option was considered before conventional technology was eliminated. According to literature about CMOs, a large $400 million conventional facility would need to produce at least 4 products to be more economically viable than outsourcing to a CMO [22]. However, when it was determined that the disposable technology method was more economical than a conventional method with an estimated investment of $40 million, the CMO became significantly less advantageous. Thus, the CMO option was also eliminated.

The final design was determined to be a disposable technology based fed-batch train. Fed batch trains followed a batch style processing, as was specified by the company, but simply added additional nutrients during processing. This improved yields in both hemagglutinin and cellular production rates [17], thus reducing the materials costs, batches, and time to produce. In the initial problem statement, it was also recommended that production scales of GlaxoSmithKline (GSK) be looked into. In the 2012/2013 season, GSK produced 21 million doses and was sold out by early January. GSK predicted it would produce 22 to 24 million doses in 2013/2014 [23]. Thus, it was assumed that 22 million doses is a reasonable production scale.

3.1.3 Media Decision

The requirements for media preparation were that the media had to be prepared from a dry powder format, was chemically defined, and serum free (animal free facility). Literature regarding the actual content of the media was limited, though present [17]. This referenced literature example, though, lacked concentration data for the formulation of a media blend that could grow cell cultures.

Most kinetic data for the growth of cellular cultures utilized commercial media, such as Sf-900 II, ExCell-405, and ExCell-420. Thus, in order to utilize this kinetic data accurately, one of the commercial media had to be used. General growth information presented in some sources, such as *Baculovirus and Insect Cell Expression* [9] had kinetic data that was initially utilized for modelling. Certain media, such as Sf-900, were only shipped in liquid form. Thus, they were immediately eliminated. The final decision fell on ExCell-420, produced by Sigma Aldrich. This line had a powder component and literature could be found regarding its growth rate [16], which was determined to be similar to the kinetic data presented in *Baculovirus and Insect Cell Expression*. Using a Multiplicity of Infection of 1.0, ample amounts of hemagglutinin could be produced, as indicated by the literature [17]. Lastly, 5 upstream sections will be utilized in order to ensure that the entire product is produced in 85 days. Approximately 48 batches were needed to produce 22 million doses, with 2 batches factored in for mistakes in the process.

3.2 Downstream Processing

There were five primary processes when it came to downstream processing: Harvest, inactivation, capture, purification, and concentration/stabilization for shipment. Each one is

discussed in its own individual section. The primary purpose of the downstream process is to recover hemagglutinin, the external viral antigen. Instead of harvesting the virus, it was determined that it would be more effective to harvest the antigen, which is the chemical the body targets during an infection.

3.2.1 Harvesting the Hemagglutinin

The first part of the process is removing the solid biomass from the stream leaving the upstream process. Based on other producers, centrifugation using a disk stack centrifuge followed by depth filtration was the most ideal process [8]. The disk stack centrifuge requires large quantities of water to run, according to SuperPro Designer v8.5, but concentrates most of the hemagglutinin in a pellet formed during the process. This step is especially helpful, as modeling data in SuperPro Designer indicated that glucose, the media, and L-glutamine were largely removed during this process. The disadvantage is that cellular biomass, host cell DNA (hcDNA), and other proteins still remained in the pellet.

The pellet was re-suspended in a buffering solution that would be utilized in the capturing process. The ratio of solution to pellet was given as 6.4g/225mL of solution [24]. The solubilized pellet was sent through a depth filtration cartridge. This step was used in order to remove the majority of the cellular biomass in the process stream. After each run, the cartridge was changed, being one of the disposable technologies employed in the downstream process.

After depth filtration, the process was then sent through a diafiltration step. The purpose of the diafiltration step is to remove excess impurities, such as glucose, L-glutamine, and media that might still be remaining in the process. From here, the process is sent to a storage tank for capturing. Diafiltration, though, does not remove hcDNA or other proteins in the process, meaning that other methods of purification are necessary.

It should be noted that in these processes, there are 3 tanks used to hold product. The tank that occurs before the centrifugation step, holds the product coming in from the upstream design. In order to keep the process on schedule, the centrifuge must run as this tank is filling. The centrifuge, though, has a lower flow rate than the rest of the processes, and thus, cannot catch up to the filling of the tank. The other two tanks are intermediate storage tanks that utilize disposable technology for their processes. This was done in order to minimize SIP and CIP procedures needed in the downstream process.

3.2.2 Inactivation of the Hemagglutinin

Because of the expression system that was selected for this process, an inactivation step was unnecessary for this process. Theoretically, "inactivation" comes from the physical removal of the hcDNA and other proteins, but this is not traditional inactivation. Two producers, Novavax [7] and Protein Sciences [8] confirm that this step is unnecessary for baculovirus insect cell expression systems. Further investigation found that this is due to the fact that baculoviruses are only infectious to arthropods [9]. Protein Sciences has also validated the product that it produces with the FDA and has proven that it is effective in humans [18]. Standard inactivation requires chemicals that are potentially harmful if left in the process, or improperly removed. Thus, eliminating this step completely from the process is highly desirable and follows the Principles

of Green Engineering [14]. Based on this overwhelming amount of information, the inactivation step can be removed from the downstream process safely.

3.2.3 Capturing the Hemagglutinin

The next step in the process is the use of an anionic exchange membrane (AEX). Other processes use an anionic exchange chromatography (AEC) or size exclusion chromatography (SEC), followed by membrane filtration for this step. It was determined that using an AEX process would be more efficient. The reason is that AEC had lower yields of hemagglutinin, according to the literature. SEC has high yields, and is effective at removing other impurities in the system. However, SEC was deemed expensive for large scale processing. Relevant information indicated that AEC had an overall yield of 80% of hemagglutinin [25], while AEX had yields of up to 86% [26]. This minor difference resulted in the need for more production runs, thus, AEX was favored.

The particular membrane used in this process is Sartobind Q. It has the capability to process 67 $L/m^2/hr$. Further investigation found that AEX removed 77% of residual protein in the process, though, no hcDNA was removed, meaning that some further purification was necessary further downstream of the process [26]. What makes AEX ideal is that AEX uses a salt based bind and elute method, similar to AEC. AEX binds proteins that flow through the membrane, while hemagglutinin and residual hcDNA pass through during the loading phase. Using a salt gradient, everything captured is eluted from the membrane. This process only requires a salt and buffer solution, which was already utilized in the process. Thus, it was again determined to be favorable as no extra chemicals are added during processing.

Pre and post storage tanks utilized in this step are disposable. This reduces the time needed to SIP and CIP for the process. It also reduces the risk of cross contamination or contamination getting into the tanks during the process.

3.2.4 Purification of the Hemagglutinin

Following the AEX process is another diafiltration step. This step removed residual salt in the process for the next chromatographic exchange process, hydrophobic interaction chromatography (HIC). HIC behaves similarly to SEC and affinity chromatography (AC). AC uses a ligand to bind the hemagglutinin until it is forced to elute the protein, making AC highly selective when it comes to binding. SEC uses the various sizes of molecules to exclude larger molecules while processing. The HIC resin chosen for this process was GE's CaptoCore 700, which exhibited these exact properties, and has been tested for hemagglutinin processing [27].

The internal ligand in the chromatography beads binds to hemagglutinin, similar to AC. At the same time, a coating around the bead has pores that exclude molecules of larger sizes, similar to SEC. Once other materials have passed through, using a higher salt concentration, the hemagglutinin is eluted from the chromatography resin. CaptoCore 700 allowed for the recovery of 94% of the hemagglutinin that was loaded onto the column. According to GE, nearly all of the residual protein was removed during this process, and ~50% of hcDNA was removed in this step. An investigation by GE found that HIC using their processing techniques and two chromatography type processes removed the majority of the hcDNA and residual proteins in the

process stream. Using an added filtration step, it was determined that hcDNA and residual proteins can be removed down to an approved level [27].

Two more holding tanks are utilized in this step. Again, these tanks are disposable for the reasons stated above. However, these holding tanks are smaller than ones utilized earlier in the process. Also, the HIC process does not use additional components, other than phosphate buffered saline, as a carrier for the hemagglutinin. Thus, the need for other chemicals in the process is eliminated, again.

3.2.5 Concentration/Stabilization Steps for Production Hemagglutinin

The final steps involve a diafiltration step that concentrates the solution. This is done in order to reduce the needed steam to freeze dry the hemagglutinin in the final step. After filtration, the system passes through an ultrafiltration and a sterile filtration step, before being freeze dried. The ultrafiltration and the sterile filtration remove nearly all of the remaining hcDNA and protein in the process that were not removed by the chromatography and other filtration steps. The literature indicates that each step yields of 97% of hemagglutinin, while removing 84% of any residual protein and 67% of the hcDNA sent through the filter in each step [28].

Before freeze drying, the final product is stored in a disposable container. At this point, more salt, phosphate buffer, and inulin sugar are added. Research indicates that the inulin acts as a stabilizing agent for storage before transportation. The recommended hemagglutinin to inulin ratio was 1:500. Stabilization of hemagglutinin lasts between 3 months to a year after freeze drying, and the potency of the virus was still viable, even when stored at 20°C. It was recommended, though, that the vaccine after preparation be stored at 4°C [29, 30].

3.2.6 Final Onsite Storage

Upon freeze drying, the entirety of the batch is stored in a single 10L bag. This unit is a dry powder that can be hydrated as needed with the other 3 parts of the vaccine in order to form the entire vaccine dose. The needed amount can be weighed out, based on the concentration of the dry power, and mixed with the other dry powder vaccine units at the formulation and filling processes so that the vaccine contains 15µg/strain/dose. The area for storage is held at a temperature of 4°C, due to the fact that this is the temperature that the vaccine unit is the most stable at [30]. By holding it at this temperature, minimal amounts of the hemagglutinin will degrade while waiting for shipment from the production facility.

4.0 Energy Balance and Utilities Requirements

The following is a summary of the utilities and energy balances for the entire process. The total of the utilities was estimated using calculation factors provided by *Novais et al* [20] along with other cost estimation factors in *Biochemical Engineering* [31].

4.1 Energy Balances

The energy flowing in and out with the streams were calculated using SuperPro. Not all mass left the system in the upstream process, which meant that the mass which remained in storage containers was considered to be lost during disposal. Once the streams were summed, pumping energy and steam energy (freeze drying) were factored in. In both cases, a positive amount of

energy resulted, which was accounted for as energy that left the processes and was not utilized during production.

Table 3: Overall Energy Balance Upstream

Overall Energy Balance Upstream	
Input on Streams (kJ/batch)	110877
Output on Sreams (kJ/batch)	-119460
Residual in Tanks (kJ/batch)	-298
Pumping Input (kJ/batch)	91800
Lost to Surroundings(kJ/batch)	-82919
Balance	0

Table 4: Overall Energy Balance Downstream

Overall Energy Balance Downstream	
Input on Streams (kJ/batch)	186591
Output on Streams (kJ/batch)	-248560
Input from Pumps (kJ/batch)	33210
Input from Steam (kJ/batch)	6416758
Loss to Surroundings (kJ/batch)	-6387998
Balance	0

It is clear from the balances that a lot of energy is lost during the freeze drying process. Further investigation should be done in order to determine if there is a way to optimize the process, or reduce the energy losses of the freeze drying unit.

4.2 Utilities

The overall utilities are summarized in the table below. As stated above, estimation was used for the energy consumption of the HVAC system, building, materials landfilled (bags, filters, etc.), and then a general contingency factor, provided by the materials referenced above.

Table 5: Upstream Utilities and Costs

Upstream			
Sections	*5*	*Batches/Section*	*10*
Process Electricity	2321.85	kW-hr/batch	
Electricity to Chill Water	2945.11	kW-hr/batch	
Water Utility for Cooling	126500	L/batch	
Includes heating, agitation, and sparging			
Chilled Water (5 Deg C and 0.5Mpa)	126500	kg/batch	
Tap Water (20C, 0.5Mpa)	84.38	kJ/kg	
Chilled Water (5 Deg C)	21.52	kJ/kg	
Pumping Usage	339.53	hr/Batch	
Pumping Power (75W, 75% Eff)	34.0	kW-hr/Batch	
Water Utility Cost	$ 3,434.48		
Electricity for Cooling	$ 7,362.77		
Electricity for Processing	$ 5,804.63		
Electricity for Pumping	$ 84.88		
Sewage	$ 31,625.00		

Table 6: Downstream Utilities and Costs

Downstream			
Sections	*1*	*Batches/Sect*	*50*
Process Electricity	102.17	kW-hr/batch	
Heating Electricity	1782	kW-hr/batch	
Water Utility for Steam	2417	L/batch	
Water Utility for Else (CIP, etc)	22596.7	L/batch	
Conversion of Water to Steam at 152 Deg. C			
Steam Heating	2417	kg/batch	
Tap Water (Deg. 20C, 0.5Mpa)	84	kJ/kg	
Sat. Water (Deg. 151.831 C)	632	kJ/kg	
Energy of Evaporation	2107	kJ/kg	
Energy Per Batch	6417	MJ/batch	
Pumping Usage	123	hr/Batch	
Pumping Power (75W, 75% Eff)	12.3	kW-hr/Batch	
Electricity for Processing	$ 255.42		
Electricity for Heating	$ 4,456.08		
Electricity for Pumping	$ 30.77		
Water Utility for Steam	$ 65.61		
Water Utility for Else	$ 613.50		
Sewage	$ 6,253.35		

Table 7: Other Utilities and Total Utilities

UV Treatment		
Liquid Upstream	6,325,000	L/yr
Liquid Downstream	1,250,669	L/yr
Liquid Process	79,553	L/yr
Total Liquid to Process	7,655,222	L/yr
UV Power to Process	7,655.22	kW-hr/yr
Electricity to Process	30,620.89	kW-hr/yr
Electricity for Processing	$ 1,531.04	
Extra Utilities to be Considered		
HEPA Vac. Air Processing		$ 30,000.00
General Electricity		$ 10,000.00
Landfill for Disposables		$ 124,000.00
Contingency (20%)		$ 51,813.93
Total Utilities		
Total Utilities		**$ 277,331.45**

5.0 Equipment List and Unit Descriptions

The following is a summary of process equipment that is needed for the production in each section. This includes both equipment and disposables. A brief explanation of each piece of equipment follows the tables. Some equipment, such as the UV pre-sewage kill unit and pumps are not included in the PFD, but are included in the equipment analysis.

5.1 Equipment Lists

The following is a list of utilized equipment for processing. Note that each table includes equipment for a single section in the process for the sake of simplicity:

Table 8: Upstream Equipment Cost Summary

Upstream Equipment per Section	
Operational Sections	**5**
Wave Bioreactor 20/50	1
Wave Bioreactor 100/200	1
XDR Disposable Reactor - 200L	1
XDR Disposable Reactor - 1000L	1
Skid for Disposable Bag - 50L	3
Skid for Disposable Bag - 1000L	1
Variable Peristaltic Pump - 3.4LPM (10% Backup)	14
Sterile Tube Fuser-Dry	1

Table 9: Downstream Equipment Cost Summary

Downstream Equipment per Section	
Operational Sections	**1**
Freeze Dryer - 39.15kg Sub. Capacity	1
Disk Stack Centrifuge - 71.36L/hr	1
Anionic Exchange Membrane	1
CC700 Chromatography Column - 12.59L	1
Diafilter - Area 1.85m2	1
Diafilter - Area 0.09m2	1
Diafilter - Area 1.22m2	1
Ultra Filter - Area 0.34m2	1
Sterile Filter - Industrial	1
Depth Filter - Industrial	1
Blending Tank - 1110L	1
Skid for Disposable Bag - 50L	2
Skid for Disposable Bag - 200L	5
UV Cleaning System	1
Variable Peristaltic Pumps - 3.4LPM (10% Backup)	15

5.2 Unit Descriptions

The following is a description of each unit in the process. It has been divided into two sections: Upstream and downstream. It should be noted that where pumping is mentioned, a peristaltic pump is used for the transfer. Generally, pumps are used where sterile filters occur (such as tanks/storage bags), other types of filters, chromatography columns, and reactors. All bags contain 0.22µm sterile filters on them to prevent contamination during processing. Transfer pumps have a 180L/hr flow rate, based on the peristaltic pumps that were selected.

5.2.1 Upstream Units

The upstream portion of the process is used to grow and infect the feedstock cells. Insect cell lines are kept at a temperature of 27°C for optimal growth [10]. The cells are diluted to concentrations of $5.00x10^5$ cells/mL and grown till they are in the upper log phase of growth at $2.77x10^6$ cells/mL, where they are then diluted back down to $5.00x10^5$ cells/mL and grown again [9].

In the upstream portion of the section, the first unit is the T-flask. This is where the 1mL cell culture is first introduced to the process using injection techniques. 19mL of media and air are added in this unit, and the reaction takes place in a shaken T-flask for 52 hours.

The process is then moved to a larger 2.2L roller bottle. This is the first process that uses a fed-batch design. In the first stage, around 90mL of media is pumped into the unit as the insect cells grow for 52 hours. Then, the media transfer rate is increased to 600mL over 52 hours as cellular growth continues for the process. Once completed, the process is pumped out of the reaction vessel and into the 20/50 wave bioreactor.

Using the fed batch design again, the process volume is increased from 610mL to 3.4L over 52 hours, followed by another fed batch reaction increasing the working volume to 18.23L over 52 hours. This is the last stage of the process before the process is split: half of the volume of the tank is sent to the infection reaction wave bioreactor for seeding, and the other half is sent to be increased in volume to the full batch size cellular volume. Before splitting, approximately 100mL of cellular solution is removed. These components concentrated to $1x10^7$ cells/mL and then split into 1mL phials and stored in the vapor head space of a liquid nitrogen freezer. These phials are used in all the other batches for the year starting in the T-Flask unit mentioned above.

The infection reaction uses a 100/200L wave bioreactor. This is the part of the process where the cells are infected with the baculovirus. Pumping the media for inoculation into the system, the working volume is increased to 50L. From there, air is added along with a phial of the baculovirus for the purpose of infecting the cells at a multiplicity of infection (MOI) of 1.0 [17]. The reaction is allowed to proceed for 120 hours, while the process is held at constant temperature, and is then cooled to 4°C for storage [32]. This process waits while the main process produces the cellular batch that is going to be infected.

From the 20/50 wave bioreactor, the other half of the process enters a small XDR 200L stirred tank bioreactor. The cellular batch is again fed over 52 hours to a volume of 50.5L. This step is important as it is the last step before the cells are passaged to the main production bioreactor. It was considered performing two growth steps in this reactor using a 500L bioreactor, but 50L is

barely 10% of the total volume, making it an inefficient use of the reactor's size. Thus, it was decided that the batch would be grown to 50L, and passaged on to the main production reactor.

The main production reactor has a total volume of 2000L and a working volume of 1000L. Normally, one does not process at less than 10% of the total volume and 5% is considered to be the absolute minimum limit. However, the fed batch process quickly adds volume to the reaction, increasing the working volume to 100L within a few hours. Thus, this is considered to be acceptable for this process. The 50L batch is grown in a fed batch style to 280L over 52 hours, and then to 950L over 21 hours. At this point, the cells are ready to have the virus introduced at an MOI of 1.0 from the infection wave bioreactor. The total working volume becomes 1000L and the infection is allowed to proceed for 72 hours, which is confirmed as the most ideal time by multiple sources [9, 17]. After this, the process is then removed from the main bioreactor and sent to the downstream process.

5.2.2 Downstream Units

The main bioreactor pumps into a large downstream tank. As the main process feeds into the tank, a pump moves fluid through a disk stack centrifuge that begins to concentrate the fluid leaving the tank. The centrifuge concentrates the cellular mass and hemagglutinin in the pellet, while the supernatant is discarded from the process [13]. The pellet is then re-suspended in the phosphate buffer saline solution (134L) that will be used in the AEX process further downstream in the storage tank following the centrifugation step. Disk stack centrifugation is the most common method for this process, which is why it is used.

It should be noted that disk stack centrifugation removes a large amount of waste in the stream that might clog downstream filters, but it is recommended that the fluid be pumped through a 0.22μm depth filter immediately afterwards to ensure that no other particulates (especially biomass) proceed further downstream [9]. It is also recommended that after these steps, a diafiltration step be used. This further removes impurities from the process, allowing for easier processing downstream [9].

From here, the extra salt and buffer solution is added to ensure that the process is at around 0.7M NaCl and 20mM phosphate buffer. Then, the solution is pumped onto the AEX membrane. Hemagglutinin passes through, while excess proteins are captured by the membrane. Then, a 1.5M NaCl and 20mM phosphate buffer solution is used to elute the components trapped on the membrane. The membrane is then cleaned with a 0.1M HCl and 2M NaCl solution followed by a 1M NaOH and 1M NaCl solution. The membrane is then sanitized/equilibriated for 1 hour using 0.5M NaOH, after which point ready to be used again [25].

Nothing is added in the AEX storage tank because for the HIC column, there is too much salt in the solution. Thus, the system is pumped through a diafiltration process and into another storage tank where phosphate buffer is added. The diafiltration in this step actually halves the total volume of the solution (134L to 67L) in order to concentrate the process, thus, reducing the volumes being processed. At this point, the salt concentration is 0.15M NaCl and more phosphate buffer is added to bring it to 20mM phosphate buffer [27, 33].

From here, the process is sent onto a CaptoCore 700 HIC column. 1.89L of CaptoCore 700 is needed to remove hcDNA and other proteins in the process on the 12.6L column. The beads are equilibriated with 0.15M NaCl and 20mM phosphate buffer solution, and then the fluid in the CC700 S&P tank are sent through the column. Other proteins and hcDNA continue through the column, while hemagglutinin is captured by the beads. Then, using a high salt concentration of 0.75M NaCl in 20mM phosphate buffer, the hemagglutinin is eluted from the beads in the column. It is then washed with 1M NaOH in 30% isopropanol solution. Afterwards, it is sanitized with 1M NaOH. Lastly, the column is equilibriated with 0.15M NaCl and 20mM phosphate buffer to prepare it for the next batch [27, 33].

The purified process is then pumped through another diafiltration device. The column reduces the total volume to 9.4L, and the diafilter removes excess salt and buffer in the solution. From there, the process passes through a 1 micron 750kDa ultrafilter that removes more hcDNA and proteins, and is then followed by another sterile filter of 0.22μm in size to remove other excess proteins, hcDNA, and any possible contaminants in the process [26].

Before freeze drying, the final buffer solution is added, and the process is diluted with roughly 30L of injection water. Adding 8.5kg of inulin sugar gives a stabilizing matrix for the hemagglutinin during the freeze drying process [29, 30]. This gives a 5mM Phosphate buffering solution with 0.7M NaCl solution. The full 46.7L (somewhat increased due to all the mass that is added) is then passed into the freeze dryer where 39.15kg of water is removed, and the final product containing hemagglutinin, NaCl, phosphate buffer, and inulin is left (~8.7kg).

The final product is then stored the shipping area in a 10L bag that is vacuum sealed. The approximate temperature is approximately 4°C for maximum stability. This is also a safety assurance, considering stability has been shown for the powdered matrix in temperatures as a high as 20°C [30]. The bulk storage has a consistent concentration which allows for easier processing in the final fill and finish part of the vaccine process. The powder of the other vaccine units can be combined with each other in proper ratios with added phosphate buffer to form a vaccine solution containing phosphate buffered saline and 15μg of each strain per dose, as described by the *World Health Organization* [12].

6.0 Equipment Specification Sheet

The following is a list of equipment and their approximate specifications based on SuperPro Designer v8.5. Notation is based on process flow sheet above.

6.1 Upstream Process Equipment

The following list contains upstream process equipment (excluding pumps and other external components).

Lab Flask

- -100mL volume
- -Working volume 50% of max volume
- -1 unit per skid rack

Roller Bottle
- -2.2L volume
- -Working volume 50% of max volume
- -1 unit per skid per rack

RBR Storage (Roller Bottle Storage)
- -1.00L volume
- -Working volume 100% of max volume
- -1 unit per skid per rack

Small WaveRctr (20/50 GE Wave Bioreactor)
- -50L volume
- -Working volume 50% of max volume
- -1 skid per container

50L Store, Infection Media, and STR Store (3x 50L Skids and Disposable Bags)
- -50L volume
- -0.22µm sterile filter
- -Working volume 50% of max volume
- -Needs to be mixing approved
- -1 skid per container

Infection Rxn (100/200 GE Wave Bioreactor)
- -200L volume
- -Working volume 50% of max volume
- -1 skid per container

Small STR (GE XDR 200L Disposable Reactor)
- -200L volume
- -Working volume 50% of max volume
- -1 skid per container

Production STR (GE XDR 1000L Disposable Reactor)
- -2000L volume
- -Working volume 50% of max volume
- -1 skid per container

1000L Store (1000L Skid and Disposable Bag)
- -1000L volume
- -0.22µm sterile filter
- -Working volume 100% of max volume
- -Needs to be mixing approved
- -1 skid per container

6.2 Downstream Process Equipment

The following list contains downstream equipment and their unit descriptions (excluding pumps and excess process units).

Upstream Store (Blending Tank)
- -1110L volume
- -Working volume 90% of max volume
- -2.334m Height
- -0.778m Diameter

UltraCent (Disk Stack Centrifuge)
- -Sigma Factor 3030.97m^2
- -Throughput 71.36L/hr

DF Storage, AEX Hold, and CC700 S&P (3x 200L Skid and Disposable Bag)
- -200L volume
- -Working volume 100% of max volume
- -Needs to be mixing approved
- -1 skid per container

Depth Filter
- -0.22micron Depth Filter Cartridge
- -10.00m^2 Area
- -5 cartridge slots/unit
- -1 working cartridge/unit

DiaFilt Storage and AEX Store (2x 200L Skid and Disposable Bag
- -200L Volume
- -Working Volume 100% of max volume
- -1 skid per container

Impurity Remove (Diafiltration)
- -1.218m^2 membrane area

AEX Process (AEX Membrane)
- -Sartobind Q 30”
- -1.964m^2 membrane area/total membrane area
- -275.000 micron membrane thickness
- -14.545 layers
- -0.540 L membrane volume/total membrane volume
- -1350.00cm^2 bed frontal area/total bed frontal area
- -4.000mm bed height
- -1.000 over design factor

De-Salting (Diafiltration)
- -1.853m^2 membrane area

CC700 Column (HIC Chromatography Column)

*Column Shape and Dimensions
-Circular Design
-1.000m height
-0.127m diameter
-1.200m max diameter
-12.59 volume

*Column Sediment Bed Dimensions
-0.150m height
-0.100m min height
-Volume 1.89L
-1.00 overdesign factor

DiaF Store (50L Skid and Disposable Bag)

-50L volume
-Working volume 100% of max volume
-1 skid per container

Concentration (Diafiltration)

-0.088m^2 membrane area

UF Final (Ultrafiltration)

-0.3380m^2 filter area
-750 kDa and 1 micron filter

Sterile Filt (Sterile Filtration)

-0.22µm filter
-10.00m^2 filter area
-5 max cartridge slots
-1 cartridge slot used

Freeze Store (50L Skid and Disposable Bag)

-50L volume
-Working volume 100% of max volume
-Needs to be mixing approved
-1 skid per container

Final Freeze (Freeze Dryer)

-39.145kg sublimation capacity
-0.491m^2 tray area

6.3 Other Process Equipment

The following is a list of process equipment that are utilized by both the upstream and downstream processes, but are not represented on the process flow diagram.

UV Cleaning System (Trojan UV Max Pro)
-10GPM Flow rate
-30mJ/cm^2 dose
-25% efficient

Peristaltic Pumps (Variable speed)
-Max flow 3.4LPM
-10% backup pumps on hand

7.0 Equipment Cost Summary

The following tables contain the equipment costs for both the individual units and the total units needed. Costing information was obtained partially from SuperPro Designer v8.5, examining GE, Pall, and Sartorius's websites, and direct contact with GE and Pall when a specific unit's price was needed [34, 35]. SuperPro Designer gave an additional estimate for components that may have been left out in the costing. Pumps were priced from Cole-Parmer's website. The UV treatment system was priced from excel water's website.

7.1 Process Fixed Equipment

This section includes costing for all non-disposable components of the equipment.

Table 10: Upstream Process Fixed Equipment

Upstream Process			
Operational Section		**5**	
Process	**Amount/Section**	**Price (USD)**	**Total (USD)**
Wave Bioreactor 20/50	1	$ 40,000.00	$ 200,000.00
Wave Bioreactor 100/200	1	$ 170,000.00	$ 850,000.00
XDR Disposable Reactor - 200L	1	$ 265,000.00	$ 1,325,000.00
XDR Disposable Reactor - 1000L	1	$ 530,000.00	$ 2,650,000.00
Skid for Disposable Bag - 50L	3	$ 407.00	$ 6,105.00
Skid for Disposable Bag - 1000L	1	$ 9,000.00	$ 45,000.00
Variable Peristaltic Pump - 3.4LPM (10% Backup)	14	$ 1,898.00	$ 132,860.00
Sterile Tube Fuser-Dry	1	$ 20,000.00	$ 100,000.00
Unlisted Equipment (by SuperPro)	1	$ 168,000.00	$ 840,000.00
Total Equipment Cost			**$ 6,148,965.00**

Table 11: Downstream Process Fixed Equipment

Downstream Process			
Operational Section		**1**	
Process	**Amount/Section**	**Price (USD)**	**Total (USD)**
Freeze Dryer - 39.15kg Sub. Capacity	1	$ 331,000.00	$ 331,000.00
Disk Stack Centrifuge - 71.36L/hr	1	$ 112,000.00	$ 112,000.00
Anionic Exchange Membrane	1	$ 2,000.00	$ 2,000.00
CC700 Chromatography Column - 12.59L	1	$ 58,000.00	$ 58,000.00
Diafilter - Area 1.85m^2	1	$ 12,627.29	$ 12,627.29
Diafilter - Area 0.09m^2	1	$ 12,627.29	$ 12,627.29
Diafilter - Area 1.22m^2	1	$ 12,627.29	$ 12,627.29
Ultra Filter - Area 0.34m^2	1	$ 12,627.29	$ 12,627.29
Sterile Filter - 10.00m^2	1	$ 25,254.58	$ 25,254.58
Depth Filter - 10.00m^2	1	$ 25,254.58	$ 25,254.58
Blending Tank - 1110L	1	$ 198,000.00	$ 198,000.00
Skid for Disposable Bag - 50L	2	$ 470.00	$ 940.00
Skid for Disposable Bag - 200L	5	$ 6,847.00	$ 34,235.00
UV Cleaning System	1	$ 2,480.00	$ 2,480.00
Variable Peristaltic Pumps - 3.4LPM (10% Backup)	15	$ 1,898.00	$ 28,470.00
Undeffined Equipment	1	$ 244,000.00	$ 244,000.00
Total Equipment Cost			**$ 1,112,143.32**

7.2 Process Disposable Equipment

The following is a summary of all disposable equipment based on a per batch basis. Costs for all disposable equipment per year are included.

Table 12: Disposable Equipment Costs for the Upstream Process

Upstream Process			
Operational Section	**5**	**Batches/Section**	**10**
Process	**Amount/Section/Batch**	**Price (USD)**	**Total (USD)**
100 mL T-Flask	1	$ 1.70	$ 85.00
2.2L Roller Bottle	1	$ 6.00	$ 300.00
Culture Bag for RB - 50L	1	$ 500.00	$ 25,000.00
Culture Bag for RB - 200L	1	$ 2,000.00	$ 100,000.00
Culture Bag for STR - 200L	1	$ 4,000.00	$ 200,000.00
Culture Bag for STR - 1000L	1	$ 10,000.00	$ 500,000.00
Small Disposable Bag - 1L	1	$ 37.00	$ 1,850.00
Disposable Mixing Bag - 50L	3	$ 138.00	$ 20,700.00
Disposable Mixing Bag - 1000L	1	$ 1,600.00	$ 80,000.00
Total Disposables Cost/Year			**$ 927,935.00**

Table 13: Disposable Equipment Costs for the Downstream Process

Downstream Process				
Operational Section	**1**	**Batches/Section**		**50**
Process	**Amount/Section/Batch**	**Usage**	**Price (USD)**	**Total (USD)**
Sartobind Q (AEX) - 30"	1	Single	$ 5,680.00	$ 284,000.00
Dft DEF Cartridge	1	Single	$ 280.00	$ 14,000.00
Disposable Mixing Bag - 200L	3	Single	$ 320.00	$ 48,000.00
Disposable Mixing Bag - 50L	1	Single	$ 1,000.00	$ 50,000.00
Disposable Storage Bag - 50L	1	Single	$ 138.00	$ 6,900.00
Disposable Storage Bag - 200L	1	Single	$ 320.00	$ 16,000.00
Sterile Filter - 0.2 micron	1	Single	$ 2,000.00	$ 100,000.00
CaptoCore700	1	Multi	$ 5,842.00	$ 5,842.00
Diafiltration Membrane	1	Multi	$ 21,341.60	$ 21,341.60
UF Membrane	1	Multi	$ 663.00	$ 663.00
UV Lamp	1	Multi	$ 245.00	$ 245.00
UV Sleeve	1	Multi	$ 64.00	$ 64.00
Total Disposables Cost/Year				**$ 547,055.60**

8.0 Fixed Capital Investment Summary

The following fixed capital investment summary was developed using the costing factors described in *Biochemical Engineering* and by *Novais et al* [20, 31]. In order to compare the two technologies, a costing evaluation was performed for a similar sized conventional technology plant. The total capital investment is included as a reference for later sections.

Table 14: Disposable Technology Fixed Capital and Total Capital Investments

Disposable Technology		
	Contingency Factor	**1.15**
Item	**Factor**	**Cost**
Equipment (with Utilities)	0.2	$ 7,071,309.75
Piping and Installation	0.33	$ 10,500,894.98
Process Control	1	$ 13,081,923.04
Instrumentation	0.66	$ 14,001,193.31
Electrical Power	1	$ 8,485,571.70
Building Works	0.8	$ 46,953,496.74
Detail Engineering	0.5	$ 13,612,271.27
Construction and Site Mang.	0.75	$ 10,606,964.63
Commisioning	1	$ 2,474,958.41
Validation	0.5	$ 18,738,970.84
Fixed Capital Investment		***$ 145,527,554.66***
Working Capital	***15% of FCI***	***$ 3,478,616.09***
Total Capital Investment		***$ 149,006,170.75***

Table 15: Conventional Technology Fixed Capital and Total Capital Investments

Conventional Technology		
	Contingency Factor	**1.15**
	Factor	**Conventional Tech**
Equipment (with Utilities)	1	$ 30,744,825.00
Piping and Installation	0.9	$ 31,820,893.88
Process Control	0.37	$ 13,081,923.04
Instrumentation	0.6	$ 21,213,929.25
Electrical Power	0.24	$ 8,485,571.70
Building Works	1.66	$ 58,691,870.93
Detail Engineering	0.77	$ 27,224,542.54
Construction and Site Mang.	0.4	$ 14,142,619.50
Commisioning	0.07	$ 2,474,958.41
Validation	1.06	$ 37,477,941.68
Fixed Capital Investment		***$ 245,359,075.91***
Working Capital	***15% of FCI***	***$ 36,803,861.39***
Total Capital Investment		***$ 282,162,937.30***

It should be noted that the total capital and fixed capital investments of the conventional technology are almost double the total and fixed capital investments of the disposable technology. It is also worthwhile to note that the working capital of the conventional technology is 10 times the working capital of the disposable technology.

9.0 Safety, Health, and Environmental Considerations

In the process of developing the plant that manufactures vaccines, there are a number of important considerations that can affect the facility. Pathogens are a constant hazard in the workplace and to the product, especially when it is necessary to remove them from the product. Likewise, ensuring that no harmful chemicals leach into the system is also critical to the process. This provides a brief examination of some of the health and safety concerns of the process. Other safety considerations (SDS, P&ID, and HAZOP) are included in Appendices F, K, and J.

9.1 Facility Layout

The facility layout is integral to the process. Materials have to move from one unit to another efficiently and quickly. Time can be easily lost due to an improper facility layout in a process. Likewise, with materials moving back and forth, it is easy for pathogens and other potential contaminants to get into the process and create issues further down in the process stream. One article provides an excellent example of a safer facility layout:

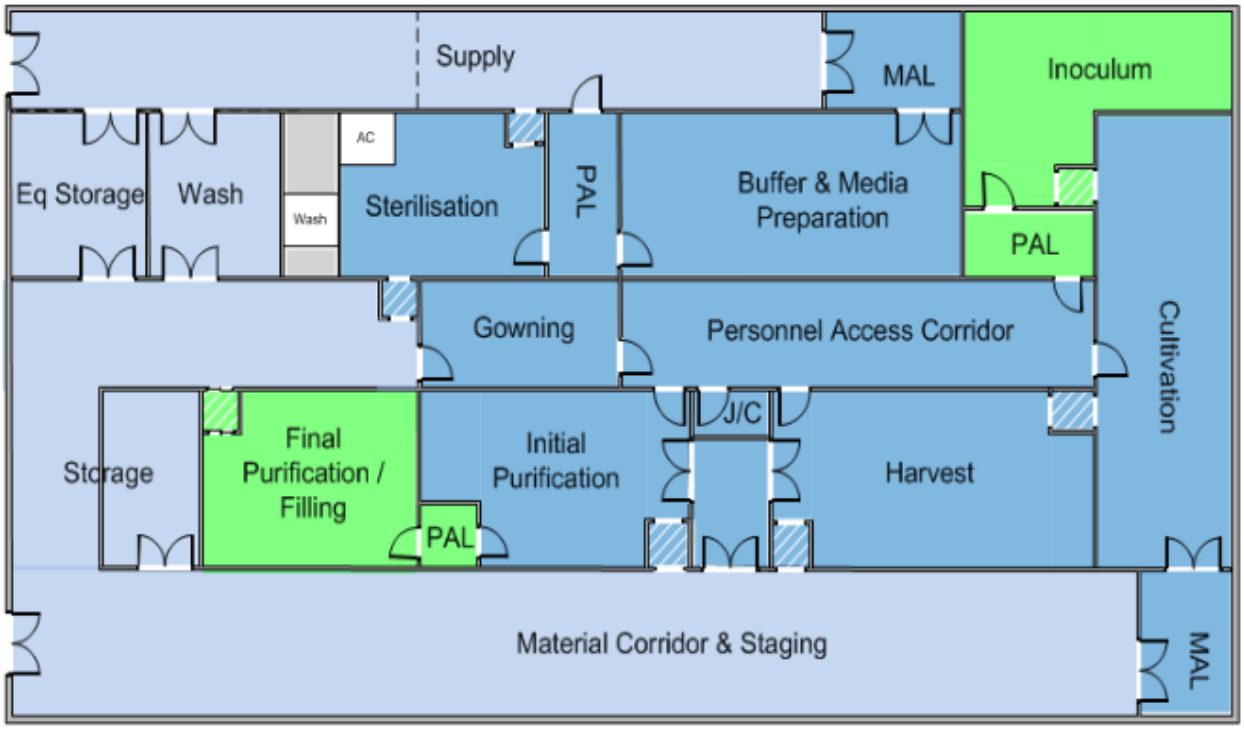

Figure 3: Theoretical Single Use Facility Layout from *Vaccine Manufacturing in the Coming Decade* [36].

The layout of this facility shows a type of modular design. Each section of the process (inoculation, culturing, purification, etc.) has its own separate stage and room in the process. Also, corridors in the top, middle, and bottom of the facility layout allow for movement between stages and areas of the facility.

Safety and health concerns are minimized by using this type of layout. While the entire facility is BSL-2, certain areas are Class D (capturing, cultivation, purification), while other portions are Class C sections of the facility (inoculum and final purification), allowing for better separation from processes where high contamination is a risk [36].

In the Class D sections of the facility, the primary portions of the downstream process and cultivation occur. This facility layout allows for direct access between each downstream process and prevents exposure of the product to other areas of the plant by having common corridors between them. This also allows for segregation of the process, should one of the parts of the process become contaminated. The added corridors allow for personnel to move in a contained environment, without having to de-gown and exit the secure portions of the facility. This means individuals can move easily from one area to another. The risk of outside contaminants entering with the individual is also reduced, as they are kept within the controlled environment.

The Class C sections are for the health and safety of the workers in the plant, while maintaining product purity. These sections can be held to more stringent standards. The reason is that the Class C portions of the facility are where the final processes and the inoculation steps that place. It is critical to prevent the baculovirus from escaping to other parts of the facility and infecting the cultivation tanks, prior to the desired infection time. Likewise, proper cleaning methods are

more strictly enforced here, as individuals carrying the virus on them could unintentionally release the baculovirus into the public and infect arthropods [9], damaging the food chain. Thus, there is also an environmental concern in ensuring that this part of the facility is stringently controlled.

Keeping outside pathogens and other materials out of the final packaging steps is also necessary. Until the package from the freeze dryer is sealed, it is desirable to isolate this part of the process from the outer areas of the plant. This part of the process occurs in the final formulation section of the plant labelled above. The actual product, once sealed, is safe to move and transport to other facilities, but until then, this part of the process must be isolated to prevent contamination.

9.2 Infection of the Staff

As stated earlier, the baculovirus expression system is not contagious to humans. The baculovirus expresses the hemagglutinin antigen that is similar to the influenza virus, but is not the influenza virus itself [7, 8, 9]. Thus, the potential for the staff at the facility to become infected is eliminated due to the selection of the cell line for the process.

There is a concern, though, of other pathogens entering the system. If one of the staff were to enter the facility while ill, they could potentially introduce an undesired pathogen into the processing system. There is no prevention for a potential infection entering the system, for reasons discussed in the next subsection, thus it would be very easy for other pathogens to enter and contaminate a batch. Individuals in the facility will be required to wear proper PPE (such as masks, gloves, gowns, facial protection, closed shoes/boots, etc.), but will also be encouraged/required to stay home should they become ill, or suspect they have become ill. This will reduce the potential for disease to enter the system.

9.3 Chemical Safety

Traditional processes use chemicals such as β-propiolactone, benzonase, and formalin in their processes. Similarly, some sources recommend the use of antibiotics when growing cellular cultures. These steps, though, are eliminated in the overall process due to their inherent risk in terms of safety for those who work with them and for the health of the people who receive the vaccine.

Looking at antibiotics, it is recommended that they are not used for processing. Some antibiotics, if improperly removed, can remain in the product. When distributed, these antibiotics can potentially cause a reaction in consumers. Secondly, antibiotics have been determined to be ineffective at eliminating pathogens that contaminate the product. According to the literature, pathogens are merely masked by antibiotics, rather than eliminated [9]. Thus, antibiotics were removed from the process for the health of those downstream due to the potential for contamination from either improperly removed antibiotics or masked pathogens.

The need for an inactivation step was eliminated due to the choice of insect cell lines for processing. Thus, traditional inactivation chemicals β-propiolactone and formalin were eliminated from the process. Both of these chemicals pose severe health hazards to workers and consumers. β-propiolactone is a suspected carcinogen, highly flammable, and can vaporize into either hazardous components or lead to tank ruptures [37]. Formalin is a mix of methanol, water,

and formaldehyde. Due to its composition, it is a Class 4 flammable and Class 3 health hazard, according to its NFPA code [38]. Both of these pose health hazards to workers as they can be vaporized and inhaled while processing, creating a potential health hazard. Factoring in their flammability, and it becomes clear that both of these components should be eliminated if possible. Lastly, if these components were improperly removed, they would potentially be injected into consumers down the product line. Thus, for the safety and health of both consumers and employees, these components were deemed unnecessary and eliminated.

Benzonase, unlike the other components, does not pose a major health hazard according to its MSDS. It is primarily used for the removal of hcDNA from the process. It does contain glycerol, which requires proper protection (such as eye and skin protection equipment), but it is otherwise non-hazardous [39]. Following the principles of green engineering, if a chemical can be eliminated, it should [14]. Benzonase cannot continue into the final product, as the glycerol could be a potential hazard to consumers if injected. Thus, by using physical means to remove the hcDNA, a benzonase treatment is eliminated from the process as well.

Strong acids and bases are used in the chromatography and membrane unit of the process. Special provisions have to be made in order to ensure the safety of employees when working with these chemicals. Neutralizing agents and face/body wash stations will be in each major room, in order to ensure that employees have the necessary means to neutralize and remove acids and bases if they are spilled. Further discussion of these chemicals is in Appendix F.

9.4 Testing and Data Logs

In order to ensure that the product is safe for injection into humans, frequent testing of the cells during their growth phase is required. The World Health Organization (WHO) has outlines for what is the proper method for certifying a batch/production line of vaccines before final distribution into the public [12]. Examples of these tests are as follows:

1. *Identity Tests*- Used to determine that the hemagglutinin is of the same type as the influenza virus for that season.
2. *Sterility Test*- Ensures that no other pathogens are contained within the lot.
3. *Hemagglutinin Content*- The WHO requires at least 12µg of each strain per dose. Most vaccines contain 15µg/strain/dose.
4. *General Safety*- Lots need to be tested to ensure that no toxins are present in the batch.
5. *Endotoxin*- Tests to ensure that no endotoxins are contained in the batch.
6. *Visual Integrity*- Any abnormal units should be discard.
7. *Records*- Proper records of processing of each batch should be kept. Improper records can result in the discarding of a batch.
8. *Retained Samples*- As part of Good Manufacturing Practices (GMP), samples of each lot should be retained for quality assurance purposes.
9. *Labelling*- The WHO outlines what information should be affixed to each carton of vaccines.
10. *Transportation*- Transportation should follow standard GMPs.
11. *Stability*- Vaccines should be studies to ensure that they are stable with time, and lack degradation during their expected lifespan
12. *Storage Conditions*- Vaccines should be stored between 2°C to 8°C.

13. *Expiration Date*- An expiration date for a vaccine should be affixed to the vaccines, and should be approved by proper authorities.

These are some of the guidelines outlined by the WHO. A sample of information that should be recorded, as outlined by the WHO, is given in Appendices H and I.

10.0 Other Important Considerations

There were other considerations that were requested by management or considered important to the project. This section discusses these considerations.

10.1 Costing and Prices

An attempt was made to accurately cost all equipment for the overall process. The reason is that economic data was then produced using multiplying factors provided by *Novais et al* [20] and *Biochemical Engineering* [31]. This included items, such as HEPA Vacuum utilities, that could not be easily quantified.

SuperPro Designer v8.5 was the initial program that was used for costing. This produced a list of all of the disposable and fixed equipment used in the process, along with usages and approximate cost data. When the initial economic analysis was performed, though, the economic data appeared too favorable to be considered reliable.

From here, manufacturing websites were visited in order to obtain up to date prices. This included visiting Pall [40], GE [41], Cole-Parmer [42], Sartorius [43], Icis [44], ATCC [45], Sigma-Aldrich [46], excel water [47], PCI-Scientific [48], and Alibaba (1 item) [49] websites over the course of the entire project. Some data for items such as Water for Injection (WFI) and tap water were provided by the American Institute for Chemical Engineers and used in the economic analysis, as requested by management.

Websites did not provide all of the relevant equipment data, though, and suppliers had to be contacted for specific items. GE [34] and Pall [35] were available for direct contact, and provided specific item data in order to improve the economic analysis. At the end of the process, only 3 items were left to the SuperPro Designer v8.5 economic analysis. SuperPro Designer v8.5 added an additional factor for unspecified equipment. For the scope of this project, that factor can include: tubing, fittings, wheel-blocks for rolling components, and any other unforeseen expenditures in the process.

Another important consideration was time to market. The comparison between the production time of a conventional facility and a disposable facility was 21 years of production. However, relevant data indicated that a disposable facility would be validated in under 2 years, while a conventional facility would take at least 4 years for validation [19]. Thus, a disposable facility was considered to have a project time line of 23 years, while a conventional had a timeline of 25 years in their respective evaluations. This turned out to play a critical role in the economic scope of the project, as is discussed in section 12 of the report.

10.2 Scheduling

One of the requirements for the cell based process is that it can produce vaccines as quickly as an egg-based process. Based on cellular data provided, it was determined that the production process for influenza vaccines using this technology is faster than egg-based technology of a similar size. One of the fastest producers is GSK with 21 million doses in 6 months [24]. The project had to produce 22 million doses in 6 months in order to be competitive.

The upstream process takes the longest time with 522.21 hours for producing a single batch, while the downstream process takes 74.8 hours to fully process a single batch. In the upstream process, the bottleneck and longest process is the Production STR with 152.32 hours, while the downstream bottleneck and longest process is the Freeze Dryer at 26 hours. This result indicates that each upstream unit can start a new batch every 152.32 hours.

In order to optimize the system, the ideal number of upstream processing units to downstream processing units was determined to be 5:1. Based on the time to process downstream, it was determined that the upstream process could not have more than 5 units (152.32hours/26hours = 5.86 units, which rounds down to 5). The following table shows the time to produce 22 million doses when compared to the number of upstream sections:

Table 16: Total Processing Time as a Function of Upstream Sections

Upstream Sections	Total Process Time (days)
1	332.7
2	177.3
3	125.4
4	99.5
5	84.0

As can be seen by the data above, the law of diminishing returns in terms of saved time becomes apparent. Using 2 processing sections yields a time just under 6 months, which for the pandemic consideration, means that 2 units was not ideal. 3 upstream sections was considered to take too long, as 1 month must be added for the time it takes to research and develop the baculovirus strain, causing this system to take almost 5.5 months. The final decision was to maximize the process and use 5 sections. The reason for this decision is that this maximizes the potential for production during a pandemic situation, which is discussed in the next subsection.

The result is that a 4 or 5 section upstream process must be utilized, which is narrowed to a 5 section upstream process in the next subsection. Scheduling allows regular production of the vaccine to have the entire product line out before any other supplier on the market (114 days, factoring in 1 month for baculovirus development). This ensures that all of the product makes it to consumers, as consumers will purchase vaccines produced by this facility before the other producers have time to put their product on the market, essentially having a temporary monopoly on the market. The first batch is ready in 21 days, allowing for rapid validation of the product in terms of time.

10.3 Pandemic Considerations

As stated above, another consideration that came into scheduling was a pandemic situation. During a pandemic, the demand for influenza vaccine significantly increases. This is seen in the following figure:

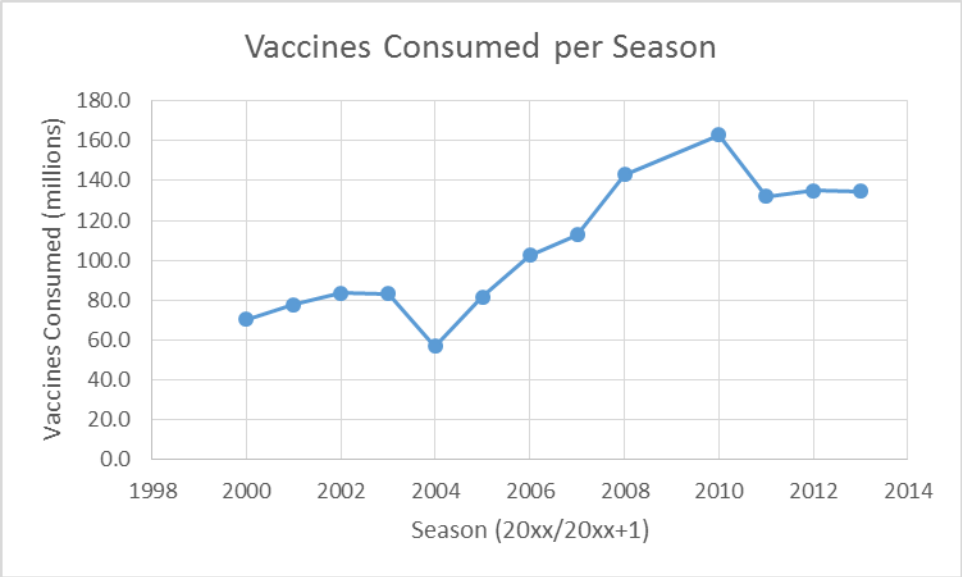

Figure 4: Vaccines Consumed per Season [1, 2, 3]

As can be seen above, there is a spike in the 2010/2011 season, when there was a suspected pandemic flu season. This lead to a spike in the amount of flu vaccine for that year of 163 million doses. That number quickly tapered off to 134.5 million doses in the 2013/2014 season [1]. Before that, the annual consumption was around 100 million or less per year [3]. This means that the doses consumed each season fluctuate positively and negatively. This shows, though, that there is a need for a facility that can quickly and effectively increase or decrease production, while still being competitive with other producers, especially during a pandemic.

In terms of a pandemic, a facility needs to produce as many vaccines as possible. The reason is that during a pandemic, the market is nearly insatiable when it comes to demand. Looking at the following table shows how having the various number of upstream sections can affect overall production over 8 months:

Table 17: Pandemic 8 Month Dose Production as a Function of Upstream Sections

Upstream Sections	Total Process Time (days)	Doses Per Month	Doses in 8 Month Pandemic
1	332.7	1,983,498	15,867,987
2	177.3	3,723,511	29,788,084
3	125.4	5,262,278	42,098,223
4	99.5	6,632,805	53,062,438
5	84.0	7,861,253	62,890,023

In this situation, it is clear that a 5 section upstream process is the most ideal. The extra unit adds roughly 10 million extra doses in an 8 month time frame. This can add up to $67.5 million

dollars in terms of sales at current market prices, over a smaller 4 section upstream process [50]. Thus, it can be inferred that ideally, a 5th upstream section is worth the capital investment for the extra ability to produce when the market demand increases significantly.

10.4 Storage and Final Formulation/Filling

The final consideration for the overall project is the storage. After the freeze drying process, the powdered vaccine subunit is stored in a vacuum sealed 10L bag before being shipped. The storage area containing the vaccine is to be held at 4°C, which is in line with the guidelines by the WHO [12], though, the vaccine is stable at temperatures of up to 20°C [30]. This will ensure that the vaccine subunit is properly stored in a conducive climate before being sent for final formulation and filling, while ensuring that minimal amounts of the vaccine degrade. The inulin sugar matrix helps to stabilize the hemagglutinin and prevent damage to the antigen during transport as well [29].

In the final formulation and filling step, the three subunits of the vaccine are combined into a single trivalent vaccine unit. This is then hydrated into a phosphate buffered saline solution of 20mM sodium phosphate buffer and roughly 0.7M NaCl in solution. In order to do this, some extra sodium phosphate buffer will need to be added to the solution to increase the molarity. The vaccines can be stored/shipped in 5mL bottles. The volumetric amount per dose is 0.5mL, meaning that each bottle would contain 10 doses.

Further research is necessary as to whether or not the vaccine should be preservative free or not. Vaccines with preservatives in them sell at a lower price, and were used for the economics portion of this cost comparison [50]. The reason is that the preservative, thimerosal, is a mercuric compound. The addition of a preservative can limit the market, due to stigmas against them. The vaccine can be distributed to younger age groups if it lacks preservative. These two reasons mean that investigating the possibility of a preservative free vaccine is worthwhile.

11.0 Manufacturing Costs

The following tables are the itemized manufacturing costs of the process described in the report. The itemized utilities requirements are summarized in section 4, Tables 5-7. Total product costs are included, as well, for the economic section of the report.

11.1 Materials Costs

The following tables show the material usage and costs of the described process.

Table 18: Upstream Materials Costs

Upstream Process				
Operational Section		**5**	**Batches/Section**	**10**
Material	**/Section/Batch**	**Units**	**Price (USD)/Unit**	**Total (USD)**
Inoculation Media	1	mL	$ 431.00	$ 431.00
Purified Air	382.966	kg	$ -	$ -
Media Powder	30.7692	kg	$ 974.03	$ 1,498,500.00
Water For Injection	999	L	$ 1.00	$ 49,950.00
Sodium Bicarbonate	0.349	kg	$ 0.20	$ 3.49
Total Material Price				**$ 1,548,884.49**

Table 19: Downstream Materials Costs

Downstream Process				
Operational Section		**1**	**Batches/Section**	**50**
Material	**/Section/Batch**	**Units**	**Price (USD)/Unit**	**Total (USD)**
Hydrochloric Acid (1M)	2.7	L	$ 26.40	$ 3,564.00
Inulin	8.5	kg	$ 20.00	$ 8,500.00
Isopropanol	2.637	kg	$ 4.87	$ 642.56
Sodium Dibase Phosphate (anhy)	0.737	kg	$ 2.05	$ 75.48
Sodium Mobase Phosphate (anhy)	0.182	kg	$ 2.17	$ 19.76
Sodium Chloride	9.707	kg	$ 0.15	$ 72.80
Sodium Hydroxide	0.435	kg	$ 21.80	$ 474.08
Water	10660	kg	$ 0.54	$ 289,426.25
Water For Injection	592	L	$ 1.00	$ 29,602.70
Total Material Price				**$ 332,377.62**

11.2 Disposables Costs

Tables 12 and 13 of section 7 summarize these costs. They are considered to be a part of the operational cost of the process, and are accounted for as the *Total Materials Cost* in Table 20.

11.3 Total Manufacturing Costs

For the economic comparison in section 12, the manufacturing costs of both a conventional process and a disposable process were calculated using factors in *Novais et al* [20] and *Bioprocess Engineering* [31]. These tables are later used in the full economic comparison.

Table 20: Total Manufacturing and Product Costs of Disposable Technology

Disposable Technology		
Item	**Factor**	**Costs**
Total Materials Cost	16	$ 3,356,252.71
Labor Costs	1	$ 489,453.52
Utilities	0.5	$ 244,726.76
Depreciation (Estimate not used)	0.6	$ 398,555.01
Other (Royalties, Licensure, etc)	1	$ 1,643,165.39
Equipment Amortization	12% Int. over 10 years	$ 1,256,531.25
Building Amortization	12% Int. over 30 years	$ 5,801,558.58
Land Tax and Insurance	4% of Fixed Capital	$ 5,821,102.19
Plant Overhead	25% of Labor Costs	$ 122,363.38
Manufacturing Costs		$ 18,735,153.77
General Expenses	11.55% of Sales	$ 17,189,865.00
Total Product Cost		***$ 35,925,018.77***
Total Product Cost 1st Year		***$ 33,879,802.28***

Table 21: Total Manufacturing and Product Costs of Conventional Technology

Conventional Technology		
Item	**Factor**	**Cost**
Total Materials Cost	0.06	$ 209,765.79
Labor Costs	0.14	$ 489,453.52
Utilities	0.14	$ 489,453.52
Depreciation (Estimate not used)	0.19	$ 664,258.35
Other (Royalties, Licensure, etc)	0.47	$ 1,643,165.39
Equipment Amortization	12% Int. over 10 years	$ 5,320,376.95
Building Amortization	12% Int. over 30 years	$ 7,251,948.22
Land Tax and Insurance	4% of Fixed Capital	$ 9,814,363.04
Plant Overhead	25% of Labor Costs	$ 122,363.38
Manufacturing		$ 25,340,889.81
General Expenses	11.55% of Sales	$ 17,189,865.00
Total Product Cost		***$ 42,530,754.81***
Total Product Cost 1st Year		***$ 41,936,418.40***

It should be noted that despite the disposable technology using more materials than the conventional technology, the conventional technology still has almost $10 million more in manufacturing costs. The manufacturing costs are all of the operational costs or the total product cost minus general expenses.

12.0 Economic Analysis

Two distinct economic analyses were requested. The first analysis requested was the price of the influenza vaccine with a discrete IRR of 25%. The second analysis was the economic value at current market prices. Revenue was based on 50% of the doses being sold in the private sector/smaller customers and 50% of the doses being sold in the public sector/larger customers, which is normal for most vaccine manufacturers [3]. Prices calculated reflect the actual market price before a $0.75/dose excise tax is assessed against the dose.

12.1 Price with an Internal Rate of Return of 25%

The following tables summarize the economics of a disposable plant design and a conventional plant of similar size based on an internal rate of return of 25%:

Table 22: Economics of Disposable Technology with an IRR of 25%

Economics of Disposable Technology at an IRR of 25%		
Public Price	$ 3.78	
Private Price	$ 7.04	
Payback Period	4.26	Years
Rate of Return	22.84%	
Avg. Annual Net	$ 37,279,362.59	

Table 23: Economics of Conventional Technology with an IRR of 25%

Economics of Conventional Technology at an IRR of 25%		
Public Price	$ 8.24	
Private Price	$ 11.50	
Payback Period	3.18	Years
Rate of Return	27.58%	
Avg. Annual Net	$ 92,655,938.68	

The production lifespan of each plant was 21 years, with the first year only producing half of the anticipated product. However, the total project life was slightly different for each plant. The conventional facility was estimated to take 4 years to build, while the disposable facility took only 2 years. According to the literature, this is the normal construction time frame for each of these facilities, as a conventional plant takes a longer time to design, commission, qualify, and validate [19].

In this comparison, the key points are the prices. The vaccines that are being produced are trivalent influenza vaccines, and it was assumed that there would be some level of preservative in each dose. Thus, the most economically unfavorable view is given for each case. By comparison, current manufacture GSK sells the lowest price trivalent influenza vaccine, Flulaval Trivalent, which is egg based and contains a preservative [51]. The price is $7.65/dose in private sale and $5.88/dose in public sale [50].

Based on these prices, the disposable technology is clearly competitive. By producing at a price below the competition, it can be almost assured that nearly all of the vaccines will be sold. By producing at a lower price than the cheapest egg based competition, the economics are clearly favorable for disposable technology. A rate of return in the 20 percent range with a payback period just over 4 years means that this project is easily viable at these prices.

The conventional technology paints a grimmer picture, by comparison. The average net earnings is three times that of the disposable technology, and the short payback period and rate of return make it deceivingly appear like the more favorable option. The prices, though, are higher than the highest egg based trivalent vaccine with preservative, Fluzone, at a price of $8.153/dose in the public sector and $10.69/dose in the private sector in a 10 dose vial [50]. This makes the conventional technology option significantly less competitive in the current market, adding a clear strike against using this technology.

12.2 Economics at Current Prices

The final comparison looks at the technology at current prices. The prices used for this portion were the GSK vaccine prices stated earlier of $7.65/dose private and $5.88/dose public [50]. This is currently the cheapest influenza vaccine price on the market. Thus, it was considered to give the most economically unfavorable view of both technologies (worst case scenario), while showing the competitiveness of a disposable technology facility design in the current economic market. Again, the expected plant lives referenced in the previous subsection are used for the IRR and NPV calculations.

Table 24: Economics of a Disposable Plant at Current Prices

Disposable Technology at Current Prices and 12% Interest		
Net Present Value	$ 277,875,122.75	
Payback Period	2.83	years
Return on Investment	34.42%	
IRR/DCFROR	33.36%	
Average Net Earnings	$ 56,174,153.52	

Table 25: Economics of a Conventional Plant at Current Prices

Conventional Technology at Current Prices and 12% Interest		
Net Present Value	$ 90,488,506.37	
Payback Period	6.03	years
Return on Investment	14.66%	
IRR/DCFROR	16.42%	
Average Net Earnings	$ 49,251,948.44	

Using the lowest market prices for an egg based vaccine with preservative, the economic analysis becomes more realistic. According *Biochemical Engineering*, an expected nominal interest rate is 12% for a recombinant protein plant [31]. Thus, this was used for the present value and net present value calculations.

At current market prices, the payback period becomes less than 3 years and the return on investment goes into the mid 30 percent range. The average net earnings show a reasonable rate of $56 million net earnings each year. The IRR and the NPV show that the project is worth going ahead. For an investment of $149 million, the NPV is $277 million. A NPV higher than the actual investment indicates that the project would add more than the value of the investment to the company's value, thus meaning that the project should go forward because the company would be significantly more valuable than its initial investment.

When looking at the discrete IRR of 33%, Peters and Timmerhaus state that this is reflective of a "new product or process in a new application" [52]. This evaluation is somewhat accurate of the technology. While the product is not "new," it is made in a new way and uses a new type of technology to produce it. The minimum for this category is 24%, according to Peters and Timmerhaus, which means that this process is well within in the needed IRR value range for this type of process. Thus, the IRR is reflective of the process and, again, indicates that the plant design is economically favorable.

The conventional technology plant gives a different look. The payback period is increase to 6 years and the return on investment is lower at just under 15%. The average net annual earnings are almost $6 million less at $49 million. The present value gives an even worse look at conventional technology. With an investment of $282 million, the NPV is only $90 million.

While this is positive, it indicates that the project returns less of an increase in value to the company, while using a higher investment than the disposable technology.

The discrete IRR given is very modest at 16.4%. According to Peters and Timmerhaus this is a "new product entering into established market, or a new technology" [52]. While the plant uses conventional technology, the process is new using insect cell lines, thus fitting into this category. It should be noted, though, that for this category, the minimum is 16%, according to Peters and Timmerhaus [52]. This means that the conventional technology option barely meets the criteria for this category, and may not stand up to a more rigorous economic analysis. The added lower NPV ultimately shows that using conventional technology for this process is not as sound of an investment.

12.3 Economic Concluding Remarks

It is clear from the analyses above that the disposable technology is the most economically viable option. At a 25% IRR, the product price is competitive with the cheapest product on the market, while the conventional option is not competitive with any product. At a 12% interest rate and current prices, it is clear that an investment in a disposable technology process would be a more sound investment over conventional technology, according to the NPV.

13.0 Conclusions and Recommendations

It is recommended that the company invest in a process that produces trivalent influenza vaccines using an insect cell baculovirus expression system. Using disposable technology instead of conventional technology, the overall process will yield a higher value added to the company, with a lower investment. The design recommended in this report uses physical means of treating the product and eliminates the need to use harmful chemicals in the process, thereby increasing its safety.

It is recommended that further investigation be performed in order to determine if the addition of a preservative is necessary. A preservative free vaccine would reduce potential downstream materials costs, while increasing the value of the product. Increasing the plant size or operational time should be considered as well. While the production for the plant is set at a medium sized facility with 22 million doses a year, a larger facility would be able to produce more in the same amount of time or using the same technology would produce more in a slightly longer season. The results would yield higher profits for the company, but the increased production could potentially flood the market.

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Appendix A: Mass and Energy Streams

The follow appendix contains the individual and total mass and the energy of all the streams based on the process flow diagrams.

A-1: Upstream Streams

Time Ref: Batch		VialCells	Air1	Media1-1	Vent1	Media Store 1	Air2	S-102	Media 2-1
Type		Raw Material	Raw Material	Raw Material	Emission	Raw Material	Raw Material		
Total Flow	g	1	0.1474	18.9337	0.1486	697.2292	1.1792	19.9337	90.3609
Temperature	°C	25	25	25	25	25	25	27	25
Pressure	bar	1.013	1.013	1.013	1.013	1.013	1.013	1.263	3.377
Liq/Sol Vol Flow	L	0.001	0	0.019	0	0.7	0	0.02	0.0907
Total Enthalpy	kJ	0.1013	0.0037	1.9209	0.0038	70.7384	0.0298	2.184	9.1677
Total Contents	g	1	0.1474	18.9337	0.1486	697.2292	1.1792	19.9337	90.3609
Ammonium		0	0	0	0	0	0	0	0
Carb. Dioxide		0	0	0	0	0	0	0	0
Cell Biomass		0	0	0	0	0	0	0.0111	0
Glucose		0	0	0.1136	0	4.1834	0	0.1134	0.5422
Haemagglutinin		0	0	0	0	0	0	0	0
KCl		0	0	0	0	0	0	0	0
KH2PO4		0	0	0	0	0	0	0	0
L-Glutamine		0	0	0.0189	0	0.6972	0	0.0188	0.0904
Lactate		0	0	0	0	0	0	0.0001	0
Media		0.0299	0	0.4506	0	16.5941	0	0.4696	2.1506
Na2HPO4		0	0	0	0	0	0	0	0
NaH2PO4		0	0	0	0	0	0	0	0
NaHCO3		0	0	0.0066	0	0.244	0	0.0066	0.0316
Nitrogen		0	0.1131	0	0.114	0	0.9046	0	0
Oxygen		0	0.0343	0	0.0346	0	0.2746	0	0
Sodium Chloride		0	0	0	0	0	0	0	0
Water		0	0	0	0	0	0	0	0
WFI		0.9701	0	18.3439	0	675.5105	0	19.314	87.5462

Time Ref: Batch		Media 2-2	Vent2	S-101	Media Store 2	Air3	Media 3-1	Media 3-2	Vent3	S-107
Type			Emission		Raw Material	Raw Material				
Total Flow	g	500.238	0	610.5316	18148.4611	25.9429	2768.9959	14780.2624	0	9079.8798
Temperature	°C	25	25	27	25	25	25	25	25	27
Pressure	bar	2.593	1.013	2.058	1.013	1.013	1.594	1.466	1.013	1.797
Liq/Sol Vol Flow	L	0.5022	0	0.6134	18.212	0	2.78	14.839	0	9.1227
Total Enthalpy	kJ	50.7524	0	66.8975	1841.2783	0.6567	280.9325	1499.5528	0	994.9084
Total Contents	g	500.238	0	610.5316	18148.4611	25.9429	2768.9959	14780.2624	0	9079.8798
Ammonium		0	0	0	0	0	0	0	0	0
Carb. Dioxide		0	0	0	0	0	0	0	0	0
Cell Biomass		0	0	0.3394	0	0	0	0	0	5.0455
Glucose		3.0014	0	3.6521	108.8908	0	16.614	88.6816	0	54.4006
Haemagglutinin		0	0	0	0	0	0	0	0	0
KCl		0	0	0	0	0	0	0	0	0
KH2PO4		0	0	0	0	0	0	0	0	0
L-Glutamine		0.5002	0	0.6054	18.1485	0	2.769	14.7803	0	9.0179
Lactate		0	0	0.0025	0	0	0	0	0	0.0375
Media		11.9057	0	14.2031	431.9334	0	65.9021	351.7702	0	211.1432
Na2HPO4		0	0	0	0	0	0	0	0	0
NaH2PO4		0	0	0	0	0	0	0	0	0
NaHCO3		0.1751	0	0.2133	6.352	0	0.9691	5.1731	0	3.1778
Nitrogen		0	0	0	0	19.9012	0	0	0	0
Oxygen		0	0	0	0	6.0416	0	0	0	0
Sodium Chloride		0	0	0	0	0	0	0	0	0
Water		0	0	0	0	0	0	0	0	0
WFI		484.6556	0	591.5158	17583.1365	0	2682.7416	14319.8572	0	8797.0573

Time Ref: Batch		Infection	Media for Infection	S-108	S-103	Media Store 3	Air4	Media 4-1	Vent4
Type		Raw Material	Raw Material			Raw Material	Raw Material		Emission
Total Flow	g	1	40657.655	40657.655	9079.8798	41853.4684	366.2936	41178.3549	427.6984
Temperature	°C	25	25	25	27	25	25	25	27
Pressure	bar	1.013	1.013	5.518	2.313	1.013	1.013	6.349	1.013
Liq/Sol Vol Flow	L	0.001	40.8	40.8192	9.1227	42	0	41.342	0
Total Enthalpy	kJ	0.1013	4124.9811	4124.9811	994.9084	4246.304	9.2715	4177.8094	11.7322
Total Contents	g	1	40657.655	40657.655	9079.8798	41853.4684	366.2936	41178.3549	427.6984
Ammonium		0	0	0	0	0	0	0	0.0212
Carb. Dioxide		0	0	0	0	0	0	0	0.0516
Cell Biomass		0	0	0	5.0455	0	0	0	0
Glucose		0	243.9459	243.9459	54.4006	251.1208	0	247.0701	0
Haemagglutinin		0	0	0	0	0	0	0	0
KCl		0	0	0	0	0	0	0	0
KH2PO4		0	0	0	0	0	0	0	0
L-Glutamine		0	40.6577	40.6577	9.0179	41.8535	0	41.1784	0
Lactate		0	0	0	0.0375	0	0	0	0
Media		0.0299	967.6522	967.6522	211.1432	996.1125	0	980.0448	0
Na2HPO4		0	0	0	0	0	0	0	0
NaH2PO4		0	0	0	0	0	0	0	0
NaHCO3		0	14.2302	14.2302	3.1778	14.6487	0	14.4124	0
Nitrogen		0	0	0	0	0	280.9904	0	328.068
Oxygen		0	0	0	0	0	85.3032	0	99.5576
Sodium Chloride		0	0	0	0	0	0	0	0
Water		0	0	0	0	0	0	0	0
WFI		0.9701	39391.1691	39391.1691	8797.0573	40549.7329	0	39895.6491	0

Time Ref: Batch		S-104	Media Store 4	Air5	Media 5-1	Media 5-2	S-109	Vent5	To Downstream
Type			Raw Material	Raw Material				Emission	
Total Flow	g	50258.1636	895933.2819	382572.4037	227598.5106	666775.1974	49738.5348	383754.2436	994368.7116
Temperature	°C	27	25	25	25	25	4	27	27
Pressure	bar	1.013	1.013	1.013	10.081	3.08	1.867	1.013	1.013
Liq/Sol Vol Flow	L	50.4951	899.07	0	228.503	669.425	49.5539	0	999.0553
Total Enthalpy	kJ	5506.9311	90898.2039	9683.5124	23091.3353	67648.6398	807.407	10492.3423	108955.8303
Total Contents	g	50258.1636	895933.2819	382572.4037	227598.5106	666775.1974	49738.5348	383754.2436	994368.7116
Ammonium		0	0	0	0	0	0	0.8185	0
Carb. Dioxide		0	0	0	0	0	0	1.997	0
Cell Biomass		27.9625	0	0	0	0	5.0455	0	554.0401
Glucose		301.1267	5375.5997	0	1365.5911	4000.6512	298.3465	0	5957.5154
Haemagglutinin		0	0	0	0	0	0	0	25.1654
KCl		0	0	0	0	0	0	0	0
KH2PO4		0	0	0	0	0	0	0	0
L-Glutamine		49.9172	895.9333	0	227.5985	666.7752	49.6756	0	987.3145
Lactate		0.2076	0	0	0	0	0.0375	0	4.2992
Media		1168.653	21323.2121	0	5416.8446	15869.2497	1178.8253	0	23096.4782
Na2HPO4		0	0	0	0	0	0	0	0
NaH2PO4		0	0	0	0	0	0	0	0
NaHCO3		17.5902	313.5766	0	79.6595	233.3713	17.408	0	348.029
Nitrogen		0	0	293478.1297	0	0	0	294383.6942	0
Oxygen		0	0	89094.274	0	0	0	89367.7339	0
Sodium Chloride		0	0	0	0	0	0	0	0
Water		0	0	0	0	0	0	0	0
WFI		48692.7065	868024.9602	0	220508.8169	646005.15	48189.1965	0	963395.8699

Time Ref: Batch		RBR Store Res.	50L Store Res.	Inf. Med. Res.	100L Store Res.	1000L Store Res.	Energy In By Pumps	Energy Lost to Surr.
Type								
Total Flow	g							
Temperature	°C							
Pressure	bar							
Liq/Sol Vol Flow	L							
Total Enthalpy	kJ	10.8183	60.793	0	68.4946	158.2288	91800	82918.86
Total Contents	g	106.6303	599.2028	0	675.1135	1559.5739		
Ammonium		0	0	0	0	0		
Carb. Dioxide		0	0	0	0	0		
Cell Biomass		0	0	0	0	0		
Glucose		0.6398	3.5952	0	4.0507	9.3574		
Haemagglutinin		0	0	0	0	0		
KCl		0	0	0	0	0		
KH2PO4		0	0	0	0	0		
L-Glutamine		0.1066	0.5992	0	0.6751	1.5596		
Lactate		0	0	0	0	0		
Media		2.5378	14.2611	0	16.0677	37.1178		
Na2HPO4		0	0	0	0	0		
NaH2PO4		0	0	0	0	0		
NaHCO3		0.0373	0.2098	0	0.2363	0.5458		
Nitrogen		0	0	0	0	0		
Oxygen		0	0	0	0	0		
Sodium Chloride		0	0	0	0	0		
Water		0	0	0	0	0		
WFI		103.3087	580.5377	0	654.0838	1510.9933		

A-2: Downstream Streams

Time Ref: Batch		From Upstream	S-113	To WM (1)	S-114	AEB Buffer	S-116	To WM (2)	S-117	S-122
Type		Raw Material				Raw Material				
Total Flow	kg	994.3687	994.3687	990.6014	3.7673	130.7544	134.5218	1.0969	133.4249	133.4249
Temperature	°C	27	27	42	42	25	25.41	25.41	25.41	25.41
Pressure	bar	1.013	10.2	1.013	1.013	1.013	3.011	1.013	3.011	2.963
Liq/Sol Vol Flow	L	999.0553	999.0551	1000.7901	3.8054	126.502	132.5958	1.0919	131.504	131.504
Total Enthalpy	kJ	111555.6295	111552.9738	172969.4596	558.8638	13417.1592	13975.9258	57.8666	13918.0592	13917.9604
Total Contents	g	994368.7116	994368.7116	990601.3671	3767.3445	130754.41	134521.7545	1096.866	133424.8885	133424.8885
Ammonium		0	0	0	0	0	0	0	0	0
Carb. Dioxide		0	0	0	0	0	0	0	0	0
Cell Biomass		554.0401	554.0401	11.0808	542.9593	0	542.9593	542.9593	0	0
Glucose		5957.5154	5957.5154	5938.3338	19.1815	0	19.1815	0.0793	19.1022	19.1022
Haemagglutinin		25.1654	25.1654	0.5033	24.6621	0	24.6621	0.102	24.5602	24.5602
HCl		0	0	0	0	0	0	0	0	0
Inulin		0	0	0	0	0	0	0	0	0
Isopropanol		0	0	0	0	0	0	0	0	0
KCl		0	0	0	0	0	0	0	0	0
KH2PO4		0	0	0	0	0	0	0	0	0
L-Glutamine		987.3145	987.3145	984.1356	3.1789	0	3.1789	0.0131	3.1657	3.1657
Lactate		4.2992	4.2992	4.2853	0.0138	0	0.0138	0.0001	0.0138	0.0138
Media		23096.4782	23096.4782	23022.114	74.3642	0	74.3642	0.3074	74.0567	74.0567
Na2HPO4		0	0	0	0	275.8177	275.8177	1.1403	274.6774	274.6774
NaH2PO4		0	0	0	0	68.0664	68.0664	0.2814	67.7849	67.7849
NaHCO3		348.029	348.029	346.9084	1.1206	0	1.1206	0.0046	1.1159	1.1159
Nitrogen		0	0	0	0	0	0	0	0	0
Oxygen		0	0	0	0	0	0	0	0	0
Sodium Chloride		0	0	0	0	5134.0719	5134.0719	21.2257	5112.8462	5112.8462
Sodium Hydroxide		0	0	0	0	0	0	0	0	0
Water		0	0	0	0	0	0	0	0	0
WFI		963395.8699	963395.8699	960294.0058	3101.8641	125276.454	128378.3181	530.7528	127847.5653	127847.5653

Time Ref: Batch		WFI In 1	Flush In 1	To WM (3)	To WM (4)	S-121	S-119	Salt/Buffer Addition	S-101	Regen Input
Type		Raw Material	Raw Material					Raw Material		Raw Material
Total Flow	kg	130.8076	69.6293	69.6293	132.4612	131.7712	131.7712	3.7039	135.4751	3.9425
Temperature	°C	25	25	25	25.53	25.53	25.53	25	25.52	25
Pressure	bar	1.013	1.013	1.013	2.963	2.963	2.965	1.013	3.05	1.013
Liq/Sol Vol Flow	L	131.504	70	70	131.5169	131.5221	131.5221	1.9276	133.4495	3.78
Total Enthalpy	kJ	13654.2186	7268.1864	7268.1864	13969.1246	13955.6595	13955.6311	230.0945	14185.6016	396.1479
Total Contents	g	130807.5596	69629.3028	69629.3028	132461.235	131771.213	131771.213	3703.9153	135475.1283	3942.54
Ammonium		0	0	0	0	0	0	0	0	0
Carb. Dioxide		0	0	0	0	0	0	0	0	0
Cell Biomass		0	0	0	0	0	0	0	0	0
Glucose		0	0	0	12.0749	7.0273	7.0273	0	7.0273	0
Haemagglutinin		0	0	0	0.4863	24.0738	24.0738	0	24.0738	0
HCl		0	0	0	0	0	0	0	0	0
Inulin		0	0	0	0	0	0	0	0	0
Isopropanol		0	0	0	0	0	0	0	0	0
KCl		0	0	0	0	0	0	0	0	0
KH2PO4		0	0	0	0	0	0	0	0	0
L-Glutamine		0	0	0	2.0011	1.1646	1.1646	0	1.1646	0
Lactate		0	0	0	0.0087	0.0051	0.0051	0	0.0051	0
Media		0	0	0	46.8128	27.2439	27.2439	0	27.2439	0
Na2HPO4		0	0	0	173.6293	101.0482	101.0482	186.4744	287.5225	0
NaH2PO4		0	0	0	42.8483	24.9367	24.9367	46.0182	70.9549	0
NaHCO3		0	0	0	0.7054	0.4105	0.4105	0	0.4105	0
Nitrogen		0	0	0	0	0	0	0	0	0
Oxygen		0	0	0	0	0	0	0	0	0
Sodium Chloride		0	0	0	3231.9352	1880.911	1880.911	3471.4227	5352.3337	210.3385
Sodium Hydroxide		0	0	0	0	0	0	0	0	143.9579
Water		0	0	0	0	0	0	0	0	0
WFI		130807.5596	69629.3028	69629.3028	128950.7331	129704.3919	129704.3919	0	129704.3919	3588.2436

Time Ref: Batch		Wash Input	AEB Elute	EQ Input	Regen Output	Wash Output	Elute Waste 1	EQ Output	S-102	S-108
Type		Raw Material	Raw Material	Raw Material						
Total Flow	kg	0.8704	2.8953	2.7567	3.9425	0.8704	2.9196	2.7567	135.4508	135.4508
Temperature	°C	25	25	25	25	25	25	25	25.52	25.52
Pressure	bar	1.013	1.013	1.013	1.013	1.013	1.013	1.013	3.05	3.049
Liq/Sol Vol Flow	L	0.7942	2.7	2.7	3.7913	0.8277	2.818	2.7453	133.4252	133.4252
Total Enthalpy	kJ	87.9651	292.4638	285.0596	396.1479	87.9651	294.6997	285.0596	14183.3657	14183.2413
Total Contents	g	870.4404	2895.2892	2756.7	3942.54	870.4404	2919.6374	2756.7	135450.7801	135450.7801
Ammonium		0	0	0	0	0	0	0	0	0
Carb. Dioxide		0	0	0	0	0	0	0	0	0
Cell Biomass		0	0	0	0	0	0	0	0	0
Glucose		0	0	0	0	0	0	0	7.0273	7.0273
Haemagglutinin		0	0	0	0	0	3.3703	0	20.7035	20.7035
HCl		2.8402	0	0	0	2.8402	0	0	0	0
Inulin		0	0	0	0	0	0	0	0	0
Isopropanol		0	0	0	0	0	0	0	0	0
KCl		0	0	0	0	0	0	0	0	0
KH2PO4		0	0	0	0	0	0	0	0	0
L-Glutamine		0	0	0	0	0	0	0	1.1646	1.1646
Lactate		0	0	0	0	0	0	0	0.0051	0.0051
Media		0	0	0	0	0	20.9778	0	6.2661	6.2661
Na2HPO4		0	5.845	0	0	0	5.845	0	287.5225	287.5225
NaH2PO4		0	1.4424	0	0	0	1.4424	0	70.9549	70.9549
NaHCO3		0	0	0	0	0	0	0	0.4105	0.4105
Nitrogen		0	0	0	0	0	0	0	0	0
Oxygen		0	0	0	0	0	0	0	0	0
Sodium Chloride		91.042	233.1432	0	210.3385	91.042	233.1432	0	5352.3337	5352.3337
Sodium Hydroxide		0	0	54.0313	143.9579	0	0	54.0313	0	0
Water		0	0	0	0	0	0	0	0	0
WFI		776.5582	2654.8586	2702.6687	3588.2436	776.5582	2654.8586	2702.6687	129704.3919	129704.3919

Time Ref: Batch		WFI In 2	Flush In 2	To WM (5)	To WM (6)	S-103	Dilution Water	Buffer Addition	S-104	EQ In.
Type		Raw Material	Raw Material				Raw Material	Raw Material		Raw Material
Total Flow	kg	132.7186	69.6293	69.6293	201.3068	66.8626	46.1586	0.2417	113.263	9.4463
Temperature	°C	25	25	25	25.74	25.74	25	25	25.44	25
Pressure	bar	1.013	1.013	1.013	3.049	3.049	1.013	1.013	2.34	1.013
Liq/Sol Vol Flow	L	133.4252	70	70	200.1715	66.7261	46.4044	0.1381	113.2694	9.4455
Total Enthalpy	kJ	13853.7013	7268.1864	7268.1864	21434.3107	7138.8854	4818.2233	3.3195	11960.3247	980.5212
Total Contents	g	132718.6056	69629.3028	69629.3028	201306.7597	66862.626	46158.63	241.738	113262.994	9446.3261
Ammonium		0	0	0	0	0	0	0	0	0
Carb. Dioxide		0	0	0	0	0	0	0	0	0
Cell Biomass		0	0	0	0	0	0	0	0	0
Glucose		0	0	0	5.7349	1.2924	0	0	1.2924	0
Haemagglutinin		0	0	0	1.0254	19.6781	0	0	19.6781	0
HCl		0	0	0	0	0	0	0	0	0
Inulin		0	0	0	0	0	0	0	0	0
Isopropanol		0	0	0	0	0	0	0	0	0
KCl		0	0	0	0	0	0	0	0	0
KH2PO4		0	0	0	0	0	0	0	0	0
L-Glutamine		0	0	0	0.9504	0.2142	0	0	0.2142	0
Lactate		0	0	0	0.0041	0.0009	0	0	0.0009	0
Media		0	0	0	5.1137	1.1524	0	0	1.1524	0
Na2HPO4		0	0	0	234.643	52.8795	0	193.8898	246.7694	20.5628
NaH2PO4		0	0	0	57.9052	13.0496	0	47.8482	60.8978	5.0745
NaHCO3		0	0	0	0.335	0.0755	0	0	0.0755	0
Nitrogen		0	0	0	0	0	0	0	0	0
Oxygen		0	0	0	0	0	0	0	0	0
Sodium Chloride		0	0	0	4367.9623	984.3714	0	0	984.3714	82.0129
Sodium Hydroxide		0	0	0	0	0	0	0	0	0
Water		0	0	0	0	0	0	0	0	0
WFI		132718.6056	69629.3028	69629.3028	196633.0856	65789.9118	46158.63	0	111948.5418	9338.6759

Time Ref: Batch		Wash In.	Elute. In	Clean In	Load Out	Clean Out	EQ/Wash Out	Elute Waste 2	S-105	S-106
Type		Raw Material	Raw Material	Raw Material						
Total Flow	kg	9.4463	9.602	8.7933	113.2441	8.7933	9.4463	9.4463	9.6209	9.6209
Temperature	°C	25	25	25	25.44	25	25	25	25	25
Pressure	bar	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.25
Liq/Sol Vol Flow	L	9.4455	9.4455	9.4455	113.2514	9.4455	9.4455	9.4455	9.4635	9.4635
Total Enthalpy	kJ	980.5212	984.2975	803.5453	11960.3206	803.5461	980.5221	980.5221	984.3015	984.3015
Total Contents	g	9446.3261	9602.03	8793.2592	113244.103	8793.2679	9446.3356	9446.3356	9620.921	9620.921
Ammonium		0	0	0	0	0	0	0	0	0
Carb. Dioxide		0	0	0	0	0	0	0	0	0
Cell Biomass		0	0	0	0	0	0	0	0	0
Glucose		0	0	0	1.2924	0	0	0	0	0
Haemagglutinin		0	0	0	0.7871	0	0	0	18.891	18.891
HCl		0	0	0	0	0	0	0	0	0
Inulin		0	0	0	0	0	0	0	0	0
Isopropanol		0	0	2636.9639	0	2636.9665	0	0	0	0
KCl		0	0	0	0	0	0	0	0	0
KH2PO4		0	0	0	0	0	0	0	0	0
L-Glutamine		0	0	0	0.2142	0	0	0	0	0
Lactate		0	0	0	0.0009	0	0	0	0	0
Media		0	0	0	1.1524	0	0	0	0	0
Na2HPO4		20.5628	20.1932	0	246.7694	0	20.5628	20.5628	20.1932	20.1932
NaH2PO4		5.0745	4.9833	0	60.8978	0	5.0745	5.0745	4.9833	4.9833
NaHCO3		0	0	0	0.0755	0	0	0	0	0
Nitrogen		0	0	0	0	0	0	0	0	0
Oxygen		0	0	0	0	0	0	0	0	0
Sodium Chloride		82.0129	402.8244	0	984.3714	0	82.013	82.013	402.8244	402.8244
Sodium Hydroxide		0	0	237.4617	0	237.462	0	0	0	0
Water		0	0	0	0	0	0	0	0	0
WFI		9338.6759	9174.0291	5918.8335	111948.5418	5918.8395	9338.6852	9338.6852	9174.0291	9174.0291

Time Ref: Batch		WFI In 3	Flush In 3	To WM (7)	To WM (8)	S-109	S-107	To WM (9)	S-110	To WM (10)	S-111	WFI for Dilution
Type		Raw Material	Raw Material									Raw Material
Total Flow	kg	9.4134	49.7352	49.7352	9.544	9.4903	9.4903	0.0011	9.4893	0.0018	9.4875	29.8411
Temperature	°C	25	25	25	25.32	25.32	25.32	25.32	25.32	25.32	25.32	25
Pressure	bar	1.013	1.013	1.013	1.25	1.25	1.251	1.013	1.251	1.013	1.251	1.013
Liq/Sol Vol Flow	L	9.4635	50	50	9.4646	9.4646	9.4646	0.0011	9.4635	0.0017	9.4618	30
Total Enthalpy	kJ	982.6071	5191.5617	5191.5617	997.4551	994.6896	994.6705	0.0558	994.6147	0.0902	994.5244	3114.937
Total Contents	g	9413.3866	49735.2162	49735.2162	9543.9642	9490.3434	9490.3434	1.086	9489.2575	1.7556	9487.5018	29841.1298
Ammonium		0	0	0	0	0	0	0	0	0	0	0
Carb. Dioxide		0	0	0	0	0	0	0	0	0	0	0
Cell Biomass		0	0	0	0	0	0	0	0	0	0	0
Glucose		0	0	0	0	0	0	0	0	0	0	0
Haemagglutinin		0	0	0	0.3741	18.517	18.517	0.5555	17.9614	0.8981	17.0634	0
HCl		0	0	0	0	0	0	0	0	0	0	0
Inulin		0	0	0	0	0	0	0	0	0	0	0
Isopropanol		0	0	0	0	0	0	0	0	0	0	0
KCl		0	0	0	0	0	0	0	0	0	0	0
KH2PO4		0	0	0	0	0	0	0	0	0	0	0
L-Glutamine		0	0	0	0	0	0	0	0	0	0	0
Lactate		0	0	0	0	0	0	0	0	0	0	0
Media		0	0	0	0	0	0	0	0	0	0	0
Na2HPO4		0	0	0	12.7646	7.4287	7.4287	0.0004	7.4283	0.0007	7.4276	0
NaH2PO4		0	0	0	3.15	1.8332	1.8332	0.0001	1.8331	0.0002	1.833	0
NaHCO3		0	0	0	0	0	0	0	0	0	0	0
Nitrogen		0	0	0	0	0	0	0	0	0	0	0
Oxygen		0	0	0	0	0	0	0	0	0	0	0
Sodium Chloride		0	0	0	254.6336	148.1908	148.1908	0.0083	148.1825	0.0134	148.1691	0
Sodium Hydroxide		0	0	0	0	0	0	0	0	0	0	0
Water		0	0	0	0	0	0	0	0	0	0	0
WFI		9413.3866	49735.2162	49735.2162	9273.042	9314.3737	9314.3737	0.5216	9313.8521	0.8433	9313.0088	29841.1298

Time Ref: Batch		Final Buffer	Inulin	S-112	Water Removed	Final Prod.	Pump Energy In	Energy Loss to Surr.	Energy In from Steam
Type		Raw Material	Raw Material						
Total Flow	kg	0.0168	8.5	47.8454	39.1454	8.7			
Temperature	°C	25	25	25.07	20	20			
Pressure	bar	1.013	1.013	15.421	15.421	15.421			
Liq/Sol Vol Flow	L	0.0096	7.2423	46.714	39.2819	7.3363			
Total Enthalpy	kJ	0.2309	422.0936	4531.7819	3268.9265	346.4386	33210	6387998	6416758
Total Contents	g	16.813	8500	47845.4446	39145.4385	8700.006			
Ammonium		0	0	0	0	0			
Carb. Dioxide		0	0	0	0	0			
Cell Biomass		0	0	0	0	0			
Glucose		0	0	0	0	0			
Haemagglutinin		0	0	17.0634	0	17.0634			
HCl		0	0	0	0	0			
Inulin		0	8500	8500	0	8500			
Isopropanol		0	0	0	0	0			
KCl		0	0	0	0	0			
KH2PO4		0	0	0	0	0			
L-Glutamine		0	0	0	0	0			
Lactate		0	0	0	0	0			
Media		0	0	0	0	0			
Na2HPO4		13.4851	0	20.9127	0	20.9127			
NaH2PO4		3.3279	0	5.1608	0	5.1608			
NaHCO3		0	0	0	0	0			
Nitrogen		0	0	0	0	0			
Oxygen		0	0	0	0	0			
Sodium Chloride		0	0	148.1691	0	148.1691			
Sodium Hydroxide		0	0	0	0	0			
Water		0	0	0	0	0			
WFI		0	0	39154.1386	39145.4385	8.7			

Appendix B: Economic Calculations

B-1: Amortization Calculations

Disposable Technology			
Equipment Amortization			
Payment at t=0		$ 7,261,108.32	
Qrtr. Per.	4	**Rate**	12%
3%	Effective Rt.	$ 314,132.81	/3 months
Annual Total Payment		$ 1,256,531.25	/year
Building Amortization			
Payment at t=0		$ 46,953,496.74	
Qrtr. Per.	4	**Rate**	12%
3%	Effective Rt.	$ 1,450,389.64	/3 months
Annual Total Payment		$ 5,801,558.58	/year

Conventional Technology			
Equipment Amortization			
Payment at t=0		$ 30,744,825.00	
Qrtr. Per.	4	**Rate**	12%
3%	Effective Rt.	$ 1,330,094.24	/3 months
Annual Total Payment		$ 5,320,376.95	/year
Building Amortization			
Payment at t=0		$ 58,691,870.93	
Qrtr. Per.	4	**Rate**	12%
3%	Effective Rt.	$ 1,812,987.06	/3 months
Annual Total Payment		$ 7,251,948.22	/year

B-2: Depreciation Calculations

Disposable Technology			
Year	Dep. % Rate	Depreciation Value (USD)	
1	10.00%	$	14,552,755.47
2	18.00%	$	26,194,959.84
3	14.40%	$	20,955,967.87
4	11.52%	$	16,764,774.30
5	9.22%	$	13,417,640.54
6	7.37%	$	10,725,380.78
7	6.55%	$	9,532,054.83
8	6.55%	$	9,532,054.83
9	6.56%	$	9,546,607.59
10	6.55%	$	9,532,054.83
11	3.28%	$	4,773,303.79

Conventional Technology			
Year	Dep. % Rate	Conventional Value (USD)	
1	10.00%	$	24,535,907.59
2	18.00%	$	44,164,633.66
3	14.40%	$	35,331,706.93
4	11.52%	$	28,265,365.55
5	9.22%	$	22,622,106.80
6	7.37%	$	18,082,963.89
7	6.55%	$	16,071,019.47
8	6.55%	$	16,071,019.47
9	0.0656	$	16,095,555.38
10	0.0655	$	16,071,019.47
11	0.0328	$	8,047,777.69

B-3: Revenue Based on Current Prices

Production		
Concentration	15	µg/dose/strain
Product per Strain	330	g
Doses	22000000	doses/yr
Private Sale	11000000	doses/yr
Price Private Sale	$ 7.65	$/dose
Public Sale	11000000	doses/yr
Price Public Sale	$ 5.88	$/dose
Total Sales	$ 148,830,000.00	
Excise Tax for Private	$ 8,250,000.00	
Excise Tax for Public	$ 8,250,000.00	
Total Excise Tax	**$ 16,500,000.00**	
Revenue for Private	$ 56,430,000.00	
Revenue for Public	$ 75,900,000.00	
Total Revenue	**$ 132,330,000.00**	

B-4: Net Present Value of Disposable Based on Current Prices

Year	Fixed Capital Investment (USD)	Working Capital (USD)	Depreciation (USD)	Sales (USD)	Production Costs wo/Dep. (USD)	Net Earnings (USD)
-2	$ (72,763,777.33)	$ -	$ -	$ -	$ -	$ -
-1	$ (72,763,777.33)	$ (3,478,616.09)	$ -	$ -	$ -	$ -
0	$ -	$ -	$ 14,552,755.47	$ 66,165,000.00	$ (33,879,802.28)	$ 11,526,087.47
1	$ -	$ -	$ 26,194,959.84	$ 132,330,000.00	$ (35,925,018.77)	$ 45,636,513.90
2	$ -	$ -	$ 20,955,967.87	$ 132,330,000.00	$ (35,925,018.77)	$ 49,041,858.68
3	$ -	$ -	$ 16,764,774.30	$ 132,330,000.00	$ (35,925,018.77)	$ 51,766,134.50
4	$ -	$ -	$ 13,417,640.54	$ 132,330,000.00	$ (35,925,018.77)	$ 53,941,771.45
5	$ -	$ -	$ 10,725,380.78	$ 132,330,000.00	$ (35,925,018.77)	$ 55,691,740.29
6	$ -	$ -	$ 9,532,054.83	$ 132,330,000.00	$ (35,925,018.77)	$ 56,467,402.16
7	$ -	$ -	$ 9,532,054.83	$ 132,330,000.00	$ (35,925,018.77)	$ 56,467,402.16
8	$ -	$ -	$ 9,546,607.59	$ 132,330,000.00	$ (35,925,018.77)	$ 56,457,942.87
9	$ -	$ -	$ 9,532,054.83	$ 132,330,000.00	$ (35,925,018.77)	$ 56,467,402.16
10	$ -	$ -	$ 4,773,303.79	$ 132,330,000.00	$ (35,925,018.77)	$ 59,560,590.33
11	$ -	$ -	$ -	$ 132,330,000.00	$ (35,925,018.77)	$ 62,663,237.80
12	$ -	$ -	$ -	$ 132,330,000.00	$ (35,925,018.77)	$ 62,663,237.80
13	$ -	$ -	$ -	$ 132,330,000.00	$ (35,925,018.77)	$ 62,663,237.80
14	$ -	$ -	$ -	$ 132,330,000.00	$ (35,925,018.77)	$ 62,663,237.80
15	$ -	$ -	$ -	$ 132,330,000.00	$ (35,925,018.77)	$ 62,663,237.80
16	$ -	$ -	$ -	$ 132,330,000.00	$ (35,925,018.77)	$ 62,663,237.80
17	$ -	$ -	$ -	$ 132,330,000.00	$ (35,925,018.77)	$ 62,663,237.80
18	$ -	$ -	$ -	$ 132,330,000.00	$ (35,925,018.77)	$ 62,663,237.80
19	$ -	$ -	$ -	$ 132,330,000.00	$ (35,925,018.77)	$ 62,663,237.80
20	$ -	$ 3,478,616.09	$ -	$ 132,330,000.00	$ (35,925,018.77)	$ 62,663,237.80

Year	Discounted Cash Flow (USD)	Present Value (USD)	Cumulative Present Value (USD)
-2	$ (72,763,777.33)	$ (72,763,777.33)	$ (72,763,777.33)
-1	$ (76,242,393.42)	$ (68,073,565.55)	$ (140,837,342.88)
0	$ 26,078,842.93	$ 20,789,893.92	$ (120,047,448.96)
1	$ 71,831,473.74	$ 51,128,224.18	$ (68,919,224.78)
2	$ 69,997,826.55	$ 44,484,884.22	$ (24,434,340.56)
3	$ 68,530,908.80	$ 38,886,278.10	$ 14,451,937.54
4	$ 67,359,411.99	$ 34,126,374.42	$ 48,578,311.96
5	$ 66,417,121.07	$ 30,043,732.60	$ 78,622,044.56
6	$ 65,999,456.99	$ 26,656,073.73	$ 105,278,118.29
7	$ 65,999,456.99	$ 23,800,065.83	$ 129,078,184.12
8	$ 66,004,550.45	$ 21,251,698.74	$ 150,329,882.86
9	$ 65,999,456.99	$ 18,973,266.77	$ 169,303,149.63
10	$ 67,812,510.22	$ 17,405,782.36	$ 186,708,931.99
11	$ 62,663,237.80	$ 14,360,796.77	$ 201,069,728.76
12	$ 62,663,237.80	$ 12,822,139.98	$ 213,891,868.74
13	$ 62,663,237.80	$ 11,448,339.26	$ 225,340,208.00
14	$ 62,663,237.80	$ 10,221,731.49	$ 235,561,939.49
15	$ 62,663,237.80	$ 9,126,545.97	$ 244,688,485.46
16	$ 62,663,237.80	$ 8,148,701.76	$ 252,837,187.22
17	$ 62,663,237.80	$ 7,275,626.57	$ 260,112,813.79
18	$ 62,663,237.80	$ 6,496,095.15	$ 266,608,908.94
19	$ 62,663,237.80	$ 5,800,084.96	$ 272,408,993.90
20	$ 66,141,853.89	$ 5,466,128.85	$ 277,875,122.75

B-5: Net Present Value of Conventional based on Current Prices

Year	Fixed Capital Investment (USD)	Working Capital (USD)	Depreciation (USD)	Sales (USD)	Production Costs wo/Dep. (USD)	Net Earnings (USD)
-4	$ (61,339,768.98)	$ -	$ -	$ -	$ -	$ -
-3	$ (61,339,768.98)	$ -	$ -	$ -	$ -	$ -
-2	$ (61,339,768.98)	$ -	$ -	$ -	$ -	$ -
-1	$ (61,339,768.98)	$ (36,803,861.39)	$ -	$ -	$ -	$ -
0	$ -	$ -	$ 24,535,907.59	$ 66,165,000.00	$ (41,936,418.40)	$ (199,761.89)
1	$ -	$ -	$ 44,164,633.66	$ 132,330,000.00	$ (42,530,754.81)	$ 29,662,497.49
2	$ -	$ -	$ 35,331,706.93	$ 132,330,000.00	$ (42,530,754.81)	$ 35,403,899.87
3	$ -	$ -	$ 28,265,365.55	$ 132,330,000.00	$ (42,530,754.81)	$ 39,997,021.77
4	$ -	$ -	$ 22,622,106.80	$ 132,330,000.00	$ (42,530,754.81)	$ 43,665,139.95
5	$ -	$ -	$ 18,082,963.89	$ 132,330,000.00	$ (42,530,754.81)	$ 46,615,582.84
6	$ -	$ -	$ 16,071,019.47	$ 132,330,000.00	$ (42,530,754.81)	$ 47,923,346.71
7	$ -	$ -	$ 16,071,019.47	$ 132,330,000.00	$ (42,530,754.81)	$ 47,923,346.71
8	$ -	$ -	$ 9,546,607.59	$ 132,330,000.00	$ (42,530,754.81)	$ 52,164,214.44
9	$ -	$ -	$ 9,532,054.83	$ 132,330,000.00	$ (42,530,754.81)	$ 52,173,673.73
10	$ -	$ -	$ 4,773,303.79	$ 132,330,000.00	$ (42,530,754.81)	$ 55,266,861.91
11	$ -	$ -	$ -	$ 132,330,000.00	$ (42,530,754.81)	$ 58,369,509.37
12	$ -	$ -	$ -	$ 132,330,000.00	$ (42,530,754.81)	$ 58,369,509.37
13	$ -	$ -	$ -	$ 132,330,000.00	$ (42,530,754.81)	$ 58,369,509.37
14	$ -	$ -	$ -	$ 132,330,000.00	$ (42,530,754.81)	$ 58,369,509.37
15	$ -	$ -	$ -	$ 132,330,000.00	$ (42,530,754.81)	$ 58,369,509.37
16	$ -	$ -	$ -	$ 132,330,000.00	$ (42,530,754.81)	$ 58,369,509.37
17	$ -	$ -	$ -	$ 132,330,000.00	$ (42,530,754.81)	$ 58,369,509.37
18	$ -	$ -	$ -	$ 132,330,000.00	$ (42,530,754.81)	$ 58,369,509.37
19	$ -	$ -	$ -	$ 132,330,000.00	$ (42,530,754.81)	$ 58,369,509.37
20	$ -	$ 36,803,861.39	$ -	$ 132,330,000.00	$ (42,530,754.81)	$ 58,369,509.37

Year	Discounted Cash Flow (USD)	Present Value (USD)	Cumulative Present Value (USD)
-4	$ (61,339,768.98)	$ (61,339,768.98)	$ (61,339,768.98)
-3	$ (61,339,768.98)	$ (54,767,650.87)	$ (116,107,419.85)
-2	$ (61,339,768.98)	$ (48,899,688.28)	$ (165,007,108.13)
-1	$ (98,143,630.37)	$ (69,856,697.54)	$ (234,863,805.67)
0	$ 24,336,145.70	$ 15,466,060.55	$ (219,397,745.12)
1	$ 73,827,131.15	$ 41,891,496.90	$ (177,506,248.23)
2	$ 70,735,606.80	$ 35,836,859.78	$ (141,669,388.45)
3	$ 68,262,387.31	$ 30,878,437.34	$ (110,790,951.11)
4	$ 66,287,246.75	$ 26,772,307.19	$ (84,018,643.92)
5	$ 64,698,546.73	$ 23,330,944.55	$ (60,687,699.36)
6	$ 63,994,366.19	$ 20,604,473.20	$ (40,083,226.16)
7	$ 63,994,366.19	$ 18,396,851.08	$ (21,686,375.08)
8	$ 61,710,822.03	$ 15,839,630.98	$ (5,846,744.10)
9	$ 61,705,728.56	$ 14,141,360.37	$ 8,294,616.27
10	$ 60,040,165.70	$ 12,285,407.45	$ 20,580,023.72
11	$ 58,369,509.37	$ 10,663,891.13	$ 31,243,914.86
12	$ 58,369,509.37	$ 9,521,331.37	$ 40,765,246.23
13	$ 58,369,509.37	$ 8,501,188.72	$ 49,266,434.95
14	$ 58,369,509.37	$ 7,590,347.07	$ 56,856,782.02
15	$ 58,369,509.37	$ 6,777,095.60	$ 63,633,877.62
16	$ 58,369,509.37	$ 6,050,978.22	$ 69,684,855.84
17	$ 58,369,509.37	$ 5,402,659.12	$ 75,087,514.96
18	$ 58,369,509.37	$ 4,823,802.79	$ 79,911,317.75
19	$ 58,369,509.37	$ 4,306,966.77	$ 84,218,284.52
20	$ 95,173,370.76	$ 6,270,221.85	$ 90,488,506.37

B-6: IRR at 25% for Disposable Technology

Some calculations were based on the sales prices and the conventional technology prices. Thus, manufacturing costs for both technologies had to be recalculated for this step.

Disposable Technology			
Item	**Factor**	**Costs**	
Total Materials Cost	16	$	3,356,252.71
Labor Costs	1	$	489,453.52
Utilities	0.5	$	244,726.76
Depreciation (Estimate not used)	0.6	$	398,555.01
Other (Royalties, Licensure, etc)	1	$	1,643,165.39
Equipment Amortization	12% Int. over 10 years	$	1,256,531.25
Building Amortization	12% Int. over 30 years	$	5,801,558.58
Land Tax and Insurance	4% of Fixed Capital	$	5,821,102.19
Plant Overhead	25% of Labor Costs	$	122,363.38
Manufacturing Costs		$	18,735,153.77
General Expenses	11.55% of Sales	$	13,750,516.75
Total Product Cost		$	32,485,670.53
Total Product Cost 1st Year		$	30,440,454.03

Conventional Technology			
Item	**Factor**	**Cost**	
Total Materials Cost	0.06	$	209,765.79
Labor Costs	0.14	$	489,453.52
Utilities	0.14	$	489,453.52
Depreciation (Estimate not used)	0.19	$	664,258.35
Other (Royalties, Licensure, etc)	0.47	$	1,643,165.39
Equipment Amortization	12% Int. over 10 years	$	5,320,376.95
Building Amortization	12% Int. over 30 years	$	7,251,948.22
Land Tax and Insurance	4% of Fixed Capital	$	9,814,363.04
Plant Overhead	25% of Labor Costs	$	122,363.38
Manufacturing		$	25,340,889.81
General Expenses	11.55% of Sales	$	13,750,516.75
Total Product Cost		$	39,091,406.57
Total Product Cost 1st Year		$	38,497,070.15

Production		
Concentration	15	µg/dose/strain
Product per Strain	330	g
Doses	22000000	doses/yr
Private Sale	11000000	doses/yr
Price Private Sale	$ 7.04	$/dose
Public Sale	11000000	doses/yr
Price Public Sale	$ 3.78	$/dose
Revenue for Private	$ 69,206,046.55	
Revenue for Public	$ 33,346,046.55	
Excise Tax for Private	$ 8,250,000.00	
Excise Tax for Public	$ 8,250,000.00	
Total Excise Tax	$ 16,500,000.00	
Total Revenue	$ 102,552,093.10	
Total Sales	$ 119,052,093.10	

Year	Fixed Capital Investment (USD)	Working Capital (USD)	Depreciation (USD)	Sales (USD)	Production Costs wo/Dep. (USD)
-2	$ (72,763,777.33)	$ -	$ -	$ -	$ -
-1	$ (72,763,777.33)	$ (3,478,616.09)	$ -	$ -	$ -
0	$ -	$ -	$ 14,552,755.47	$ 51,276,046.55	$ (33,879,802.28)
1	$ -	$ -	$ 26,194,959.84	$ 102,552,093.10	$ (35,925,018.77)
2	$ -	$ -	$ 20,955,967.87	$ 102,552,093.10	$ (35,925,018.77)
3	$ -	$ -	$ 16,764,774.30	$ 102,552,093.10	$ (35,925,018.77)
4	$ -	$ -	$ 13,417,640.54	$ 102,552,093.10	$ (35,925,018.77)
5	$ -	$ -	$ 10,725,380.78	$ 102,552,093.10	$ (35,925,018.77)
6	$ -	$ -	$ 9,532,054.83	$ 102,552,093.10	$ (35,925,018.77)
7	$ -	$ -	$ 9,532,054.83	$ 102,552,093.10	$ (35,925,018.77)
8	$ -	$ -	$ 9,546,607.59	$ 102,552,093.10	$ (35,925,018.77)
9	$ -	$ -	$ 9,532,054.83	$ 102,552,093.10	$ (35,925,018.77)
10	$ -	$ -	$ 4,773,303.79	$ 102,552,093.10	$ (35,925,018.77)
11	$ -	$ -	$ -	$ 102,552,093.10	$ (35,925,018.77)
12	$ -	$ -	$ -	$ 102,552,093.10	$ (35,925,018.77)
13	$ -	$ -	$ -	$ 102,552,093.10	$ (35,925,018.77)
14	$ -	$ -	$ -	$ 102,552,093.10	$ (35,925,018.77)
15	$ -	$ -	$ -	$ 102,552,093.10	$ (35,925,018.77)
16	$ -	$ -	$ -	$ 102,552,093.10	$ (35,925,018.77)
17	$ -	$ -	$ -	$ 102,552,093.10	$ (35,925,018.77)
18	$ -	$ -	$ -	$ 102,552,093.10	$ (35,925,018.77)
19	$ -	$ -	$ -	$ 102,552,093.10	$ (35,925,018.77)
20	$ -	$ 3,478,616.09	$ -	$ 102,552,093.10	$ (35,925,018.77)

Net Earnings (USD)	Discounted Cash Flow (USD)	Present Value (USD)	Cumulative Present Value (USD)
$ -	$ (72,763,777.33)	$ (72,763,777.33)	$ (72,763,777.33)
$ -	$ (76,242,393.42)	$ (60,993,914.74)	$ (133,757,692.06)
$ 1,848,267.72	$ 16,401,023.19	$ 10,496,654.84	$ (123,261,037.22)
$ 26,280,874.41	$ 52,475,834.25	$ 26,867,627.14	$ (96,393,410.09)
$ 29,686,219.19	$ 50,642,187.06	$ 20,743,039.82	$ (75,650,370.26)
$ 32,410,495.02	$ 49,175,269.31	$ 16,113,752.25	$ (59,536,618.02)
$ 34,586,131.96	$ 48,003,772.50	$ 12,583,900.94	$ (46,952,717.08)
$ 36,336,100.80	$ 47,061,481.58	$ 9,869,508.02	$ (37,083,209.06)
$ 37,111,762.67	$ 46,643,817.50	$ 7,825,534.01	$ (29,257,675.04)
$ 37,111,762.67	$ 46,643,817.50	$ 6,260,427.21	$ (22,997,247.83)
$ 37,102,303.38	$ 46,648,910.96	$ 5,008,888.67	$ (17,988,359.16)
$ 37,111,762.67	$ 46,643,817.50	$ 4,006,673.41	$ (13,981,685.74)
$ 40,204,950.84	$ 48,456,870.73	$ 3,329,930.80	$ (10,651,754.94)
$ 43,307,598.31	$ 43,307,598.31	$ 2,380,860.40	$ (8,270,894.55)
$ 43,307,598.31	$ 43,307,598.31	$ 1,904,688.32	$ (6,366,206.23)
$ 43,307,598.31	$ 43,307,598.31	$ 1,523,750.65	$ (4,842,455.58)
$ 43,307,598.31	$ 43,307,598.31	$ 1,219,000.52	$ (3,623,455.05)
$ 43,307,598.31	$ 43,307,598.31	$ 975,200.42	$ (2,648,254.64)
$ 43,307,598.31	$ 43,307,598.31	$ 780,160.33	$ (1,868,094.30)
$ 43,307,598.31	$ 43,307,598.31	$ 624,128.27	$ (1,243,966.03)
$ 43,307,598.31	$ 43,307,598.31	$ 499,302.61	$ (744,663.42)
$ 43,307,598.31	$ 43,307,598.31	$ 399,442.09	$ (345,221.33)
$ 43,307,598.31	$ 46,786,214.40	$ 345,221.33	$ 0.00

B-7: IRR at 25% for Conventional Technology

Some calculations were based on the sales price. Thus, manufacturing costs for conventional technology had to be recalculated for this step.

Conventional Technology			
Item	**Factor**	**Cost**	
Total Materials Cost	0.06	$	209,765.79
Labor Costs	0.14	$	489,453.52
Utilities	0.14	$	489,453.52
Depreciation (Estimate n	0.19	$	664,258.35
Other (Royalties, Licensu	0.47	$	1,643,165.39
Equipment Amortization	12% Int. over 10 years	$	5,320,376.95
Building Amortization	12% Int. over 30 years	$	7,251,948.22
Land Tax and Insurance	4% of Fixed Capital	$	9,814,363.04
Plant Overhead	25% of Labor Costs	$	122,363.38
Manufacturing		$	25,340,889.81
General Expenses	11.55% of Sales	$	25,090,531.29
Total Product Cost		$	50,431,421.10
Total Product Cost 1st Year		$	49,837,084.69

Production		
Concentration	15	µg/dose/strain
Product per Strain	330	g
Doses	22000000	doses/yr
Private Sale	11000000	doses/yr
Price Private Sale	$ 11.50	$/dose
Public Sale	11000000	doses/yr
Price Public Sale	$ 8.24	$/dose
Revenue for Private	$ 118,297,018.57	
Revenue for Public	$ 82,437,018.57	
Excise Tax for Privat	$ 8,250,000.00	
Excise Tax for Public	$ 8,250,000.00	
Total Excise Tax	$ 16,500,000.00	
Total Revenue	$ 200,734,037.15	
Total Sales	$ 217,234,037.15	

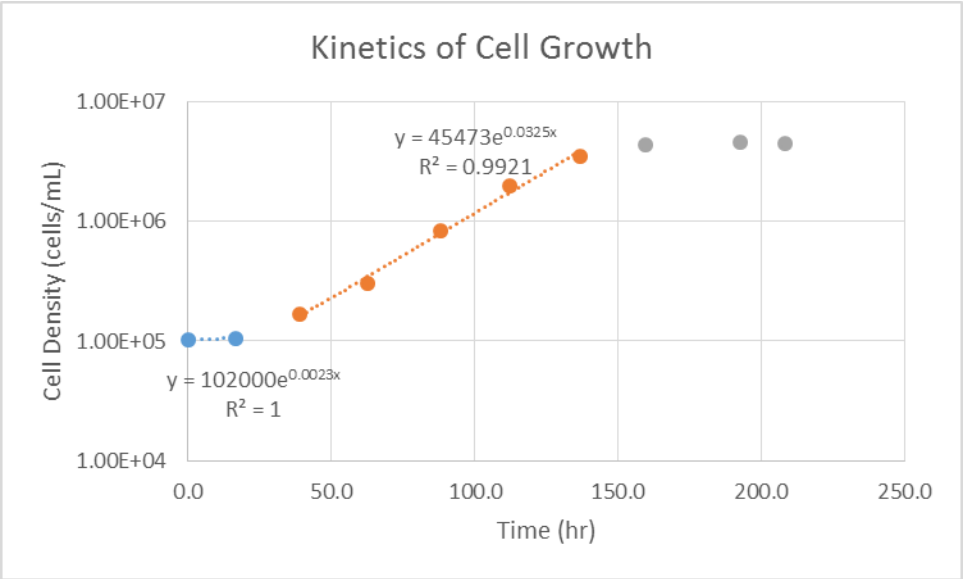
Year	Fixed Capital Investment (USD)	Working Capital (USD)	Depreciation (USD)	Sales (USD)	Production Costs wo/Dep. (USD)
-4	$ (61,339,768.98)	$ -	$ -	$ -	$ -
-3	$ (61,339,768.98)	$ -	$ -	$ -	$ -
-2	$ (61,339,768.98)	$ -	$ -	$ -	$ -
-1	$ (61,339,768.98)	$ (36,803,861.39)	$ -	$ -	$ -
0	$ -	$ -	$ 24,535,907.59	$ 100,367,018.57	$ (41,936,418.40)
1	$ -	$ -	$ 44,164,633.66	$ 200,734,037.15	$ (42,530,754.81)
2	$ -	$ -	$ 35,331,706.93	$ 200,734,037.15	$ (42,530,754.81)
3	$ -	$ -	$ 28,265,365.55	$ 200,734,037.15	$ (42,530,754.81)
4	$ -	$ -	$ 22,622,106.80	$ 200,734,037.15	$ (42,530,754.81)
5	$ -	$ -	$ 18,082,963.89	$ 200,734,037.15	$ (42,530,754.81)
6	$ -	$ -	$ 16,071,019.47	$ 200,734,037.15	$ (42,530,754.81)
7	$ -	$ -	$ 16,071,019.47	$ 200,734,037.15	$ (42,530,754.81)
8	$ -	$ -	$ 9,546,607.59	$ 200,734,037.15	$ (42,530,754.81)
9	$ -	$ -	$ 9,532,054.83	$ 200,734,037.15	$ (42,530,754.81)
10	$ -	$ -	$ 4,773,303.79	$ 200,734,037.15	$ (42,530,754.81)
11	$ -	$ -	$ -	$ 200,734,037.15	$ (42,530,754.81)
12	$ -	$ -	$ -	$ 200,734,037.15	$ (42,530,754.81)
13	$ -	$ -	$ -	$ 200,734,037.15	$ (42,530,754.81)
14	$ -	$ -	$ -	$ 200,734,037.15	$ (42,530,754.81)
15	$ -	$ -	$ -	$ 200,734,037.15	$ (42,530,754.81)
16	$ -	$ -	$ -	$ 200,734,037.15	$ (42,530,754.81)
17	$ -	$ -	$ -	$ 200,734,037.15	$ (42,530,754.81)
18	$ -	$ -	$ -	$ 200,734,037.15	$ (42,530,754.81)
19	$ -	$ -	$ -	$ 200,734,037.15	$ (42,530,754.81)
20	$ -	$ 36,803,861.39	$ -	$ 200,734,037.15	$ (42,530,754.81)

Year	Net Earnings (USD)	Discounted Cash Flow (USD)	Present Value (USD)	Cumulative Present Value (USD)
-4	$ -	$ (61,339,768.98)	$ (61,339,768.98)	$ (61,339,768.98)
-3	$ -	$ (61,339,768.98)	$ (49,071,815.18)	$ (110,411,584.16)
-2	$ -	$ (61,339,768.98)	$ (39,257,452.15)	$ (149,669,036.31)
-1	$ -	$ (98,143,630.37)	$ (50,249,538.75)	$ (199,918,575.05)
0	$ 22,031,550.18	$ 46,567,457.77	$ 19,074,030.70	$ (180,844,544.35)
1	$ 74,125,121.64	$ 118,289,755.30	$ 38,761,187.02	$ (142,083,357.33)
2	$ 79,866,524.01	$ 115,198,230.94	$ 30,198,525.05	$ (111,884,832.28)
3	$ 84,459,645.91	$ 112,725,011.46	$ 23,640,148.32	$ (88,244,683.96)
4	$ 88,127,764.10	$ 110,749,870.90	$ 18,580,745.06	$ (69,663,938.90)
5	$ 91,078,206.99	$ 109,161,170.88	$ 14,651,364.34	$ (55,012,574.56)
6	$ 92,385,970.86	$ 108,456,990.33	$ 11,645,480.66	$ (43,367,093.89)
7	$ 92,385,970.86	$ 108,456,990.33	$ 9,316,384.53	$ (34,050,709.36)
8	$ 96,626,838.59	$ 106,173,446.17	$ 7,296,183.66	$ (26,754,525.70)
9	$ 96,636,297.88	$ 106,168,352.71	$ 5,836,666.92	$ (20,917,858.78)
10	$ 99,729,486.05	$ 104,502,789.85	$ 4,596,081.30	$ (16,321,777.48)
11	$ 102,832,133.52	$ 102,832,133.52	$ 3,618,084.05	$ (12,703,693.43)
12	$ 102,832,133.52	$ 102,832,133.52	$ 2,894,467.24	$ (9,809,226.19)
13	$ 102,832,133.52	$ 102,832,133.52	$ 2,315,573.79	$ (7,493,652.40)
14	$ 102,832,133.52	$ 102,832,133.52	$ 1,852,459.03	$ (5,641,193.37)
15	$ 102,832,133.52	$ 102,832,133.52	$ 1,481,967.23	$ (4,159,226.14)
16	$ 102,832,133.52	$ 102,832,133.52	$ 1,185,573.78	$ (2,973,652.36)
17	$ 102,832,133.52	$ 102,832,133.52	$ 948,459.02	$ (2,025,193.34)
18	$ 102,832,133.52	$ 102,832,133.52	$ 758,767.22	$ (1,266,426.12)
19	$ 102,832,133.52	$ 102,832,133.52	$ 607,013.78	$ (659,412.34)
20	$ 102,832,133.52	$ 139,635,994.90	$ 659,412.34	$ (0.00)

Appendix C: Growth Kinetics and Reactor Design

C-1: Growth Rate Data [9]

Kinetics Growth Data	
Time (h)	**Cell Density (Cells/mL)**
0.0	1.02E+05
16.5	1.06E+05
39.0	1.67E+05
62.5	3.05E+05
88.0	8.37E+05
112.0	1.99E+06
136.5	3.46E+06
159.5	4.32E+06
192.5	4.56E+06
208.0	4.47E+06



Log Phase	y	=	45473	*	exp	(	0.0325	x)
Lag Phase	y	=	102000	*	exp	(	0.00023	x)
x is time (hr)	y is cell density (cells/mL)			Target Conc.			2.77E+06	

C-2: Seeding Calculations

Seeding Calculations								
	Starting Vial	Thaw	End Lag Phase	End Thaw	1st Pass	End 1st Pass	2nd Pass	End 2nd Pass
Concentration (cell/mL)	1.0E+07	5.00E+05	5.03E+05	2.77E+06	5.00E+05	2.77E+06	5.00E+05	2.77E+06
Volume (mL)	1.0	20.0	20.0	20.0	110.72	110.720	612.946	612.9
Time of Phase (hr)	0	0	25.0	52.5	0	52.7	0	52.7
Total Time (hr)	0	0	25.0	77.51	77.51	130.17	130.17	182.82
Cell Mass (g)			0.0020	0.0111		0.0613		0.3393
Glucose Consumed (g)			0.0012	0.0051		0.0282		0.1561
L-Glutamine Cons. (g)			0.0009	0.0038		0.0212		0.1172
CO2 produced (g)			0.0000	0.0000		0.0001		0.0004
O2 consumed (g)			0.0015	0.0060		0.0335		0.1853
Lactate (g)			0.0015	0.0063		0.0349		0.1932
Ammonium Ion (g)			0.0001	0.0003		0.0017		0.0000
Other Consumed (g)			0.0000	0.0007		0.0041		0.0225
Unity			0.0000	0.0020		0.0111		0.0518
% Error of Mass Balance			-0.3%	6.0%		6.0%		5.1%

Seeding Calculations									
Change in Vol. Scale	**3rd Pass**	**End 3rd Pass**	**4th Pass**	**End 4th Pass**	**5th Pass**	**End 5th Pass**	**6th Pass**	**End 6th Pass**	**Bio Reactor**
Concentration (cell/mL)	5.00E+05	2.77E+06	5.00E+05	2.77E+06	5.00E+05	2.77E+06	5.00E+05	2.77E+06	8.16E+05
Volume (L)	3.39	3.39	18.23	18.232	50.5	50.5	279.575	279.6	949
Time of Phase (hr)	0	52.65	0	52.65	0	52.68	0	52.68	0
Total Time (hr)	182.82	235.48	235.48	288.13	288.13	340.81	340.81	393.483	393.483
Cell Mass (g)		1.88		10.09		27.96		154.8848	154.88
Glucose Consumed (g)		0.86		4.64		12.87		71.27	0.00
L-Glutamine Cons. (g)		0.65		3.49		9.66		53.51	0.00
CO2 produced (g)		0.00		0.01		0.03		0.18	0.00
O2 consumed (g)		1.03		5.51		15.27		84.62	0.00
Lactate (g)		1.07		5.75		15.92		88.21	0.00
Ammonium Ion (g)		0.05		0.29		0.79		4.40	0.00
Other Consumed (g)		0.12		0.67		1.85		10.26	0.00
Unity		0.340		1.827		5.056		28.011	
% Error of Mass Balance		6.00%		6.00%		5.99%		5.99%	

	Infection Line	End Line
Volume Initial (L)	9.1	
Volume Final (L)	50.0	
Conc. (cells/L)	5.05E+05	
Time (hr)	0	120
Time to Inoculation (hr)		6.64

	Total Produced/Consumed
Cell Mass (g)	154.885
Glucose Consumed (g)	83.406
L-Glutamine Cons. (g)	62.615
CO2 produced (g)	0.210
O2 consumed (g)	99.017
Lactate (g)	103.221
Ammonium Ion (g)	5.136
Other Consumed (g)	12.008
Unity	6.406
% Error of Mass Balance	1.23%

C-3: Production Bioreactor Calculations

Production Bioreactor			
Change in Vol. Scale	**Bio Reactor**	**Growth to Infection**	**Harvest**
Concentration (cell/mL)	8.16E+05	1.63E+06	2.77E+06
Volume (L)	949	949	999
Time of Phase (hr)	0.00	21.3	72
Total Time (hr)	393.5	414.8	486.77
Cell Mass (g)	154.9	309.4	554.01686
Glucose Consumed (g)	0.0		1716.5
L-Glutamine Cons. (g)	0.0		1288.6
CO2 produced (g)	0.000		4.3
O2 consumed (g)	0.0		2037.8
Lactate (g)	0.0		2124.3
Ammonium Ion (g)	0.00		105.9
Other Consumed (g)	0.00		247.1
Haemagglutinin (g)			25.20777
Cell Mass Remainder			528.809
Unity	50.044		
% Error of Mass Balance	12.10%		

C-4: Calculation of Runs per Year Based on Strain Type [17]

	Process 1 (In. A)	Process 2 (In. A)	Process 2 (In. B)
Conversion (mg/10^9 Cells)	9.1	11.4	15.5
HA Estimate (g/batch)	25.21	31.58	42.94
HA Start (g/batch)	25.17	31.53	42.86
Percent Difference (%)	-0.168%	-0.168%	-0.168%
HA Finish (g/batch)	17.063	21.38	29.06
Effective Yeild (%)	67.80%	67.80%	67.80%
Total Batches (per strain)	19.34	15.44	11.35
Normallized	20	16	12
Total Runs/Year	48		

It was noted that different strains have different production yields. Thus, calculations were done to determine the effective overall yield of the process. Then, feeding the conversion number back into the production reactor calculation, a new total yield was produced, and the downstream effective yield determined the overall hemagglutinin content per batch. Based on 330g per strain, this was used to determine the number of batches each year.

Appendix D: Other Information

D-1: UV Reactor/Lamp Design [47]

To Kill Influenza	
6600	µWs/cm^2
0.02376	kWhr/cm^2
6.6	mJ/cm^2

Trojan UVMax Max Pro		
Flow	10	GPM
Flow	54510	LPD
Dose	30	mJ/cm^2

Energy Conversion			
1000mJ/cm^2	=	1	kWhr/m^3
	=	0.001	kWhr/L

D-2: Data Table of Market Information

Market Trends for Dose Distribution		
Season	**Doses Distributed (millions)**	**Source**
2000-01	70.4	[3]
2001-02	77.7	[3]
2002-03	83.5	[3]
2003-04	83.1	[3]
2004-05	57.0	[3]
2005-06	81.5	[3]
2006-07	102.5	[3]
2007-08	112.8	[3]
2008-09	143	[2]
2010-11	163.0	[1]
2011-12	132.1	[1]
2012-13	134.9	[1]
2013-14	134.5	[1]

Appendix E: Explanation of Calculations

E-1: Kinetic Growth Rates

An equation was supplied in *Baculovirus* [9] that calculated the growth rate of cells as a function of time. The equation is the following:

$$\boldsymbol{N = N_0 * \exp(\mu * t)}$$

Where t is time, N_0 is the cell count at the start, N is the cell count at the end of the time, and μ is the specific growth rate $0.0325 hr^{-1}$. N_0 was $5.00*10^5$ cells/mL, and N was the target concentration of $2.77*10^6$ cells/mL. Time was found using an Excel "What-If" analysis for each reaction step. Once the target concentration was found, the new volume was found with the following equation:

$$\boldsymbol{V = \frac{N}{N_0} * V_0}$$

Where V is the working volume of the next reactor and V_0 is the old working volume. After the third passage calculation, the volume was converted from mL to L. Using these equations, the time of each growth phase could be calculated based on the higher end of the logarithmic growth phase. Ideally, the working volume was between 10% and 50% of the overall reactor volume, and passages were processed in the same reactor to reduce the total amount of reactors needed for each phase of the process.

The exception is the production reactor, where the initial working volume is just above 5% of the total volume. However, the working volume reaches 10% of the total volume within a few hours.

E-2: Calculation of Consumption/Production Rates

Numerous sources stated that there were many components consumed or produced during the process. *Baculovirus* stated that while items such as CO_2, ammonium, and lactate were produced, they were not produced at such a rate to inhibit growth of the insect cells [9]. These items, along with the consumption of the primary components L-glutamine, glucose, and oxygen, were calculated in their production/consumption rates using the following equation:

$$\boldsymbol{r_A = k_A * c_A}$$

This is the simple rate law that was assumed. However, in the given rates, k, for the above compounds, the production/consumption is dependent upon time and cellular concentration [15, 16] using the units mass/cells/time. Thus, the rate law is modified to the following:

$$\boldsymbol{r_A = k_A * N}$$

This derivation becomes useful in the calculation of the production/consumption of a given chemical. It converts the rate into a function of time, *t*. A problem is seen, though, in trying to determine the total amount produced/consumed in each process:

$$r_A = k_A * N_0 * \exp(\mu * t)$$

In order to calculate the amount of mass produced, the term must be integrated from time 0 to the final time *t*. By multiplying both sides by *dt* (essentially 1 or dt/dt on one side), and then integrating, a new equation is obtained:

$$c_A = (k_A * \frac{N_0}{\mu} * \exp(\mu * t) - k_A * \frac{N_0}{\mu})$$

Merging the other side and multiplying by the final volume of each passage, the total mass is calculated. This yields the following final equation:

$$m_A = k_A * \frac{N_0}{\mu} * (\exp(\mu * t) - 1) * V$$

This is the generic final equation used to calculate the production or consumption of a given component. There is an interesting note about the equation above: the given rate law does not have an inherent negative in the front, as is normal with rate laws. The reason is that cells were produced during the reaction, and due to the cellular production rate, a cancelling negative would not have been yielded. This meant that the rate constant had to be multiplied by the integer -1 or 1, depending on if the component was consumed or produced. If the component was produced, then it was multiplied by 1. If the component was consumed, then the rate constant was multiplied by -1. Thus, the positive or negative value reflected what side of the reaction the component fell on. The kinetic constants are summarized in the following table:

Kinetic Constants	
k for glucose cons (g/cell/hr)	3.65E-12
k for glutamine cons (g/cell/hr)	2.74E-12
k for carbon dioxide (g/cell/hr)	9.17E-15
k for oxygen (g/cell/hr)	4.33E-12
k for lactate (g/cell/hr)	4.52E-12
k for ammonium (g/cell/hr)	2.25E-13
k for other (g/cell/hr)	5.25E-13

In the full reported table in Appendix C, the absolute value of all the products was taken, yielding positive values for all the masses. In this case, the usage or production of an item was noted in the side. All other components consumed were put into one main term and a single rate constant for their consumption was derived using Excel "Solver" and the overall mass balance being as close to 0 as possible. The error of the overall mass balance being 1.23% was deemed acceptable for the calculations. SuperPro v8.5 did not agree with the production/consumption of

the amount calculated. This was not reconciled and could be due to the media consumption term in SuperPro being too high or too low.

E-3: Infection Production

The amount of hemagglutinin produced was based on a mass per cellular amount. It was calculated using a simple equation:

$$m_{virus} = c_{cells} * \frac{1000mL}{1L} * \frac{\frac{V}{10^9}}{\frac{1000mg}{g}} * 9.1$$

Where 9.1 is the milligrams of hemagglutinin produced per 10^9 cells [17]. The various values found were used to find this value in each step. The total yield was calculated based on the effective yield predicted by SuperPro and the literature. This was performed for each strain, with 9.1 being the lowest value.

E-4: Simple Economic Calculations

Factors used to calculate the value of the various components of disposable technology given were based on the value of conventional equipment [20]. Thus, using the value of the disposable equipment, the value of the conventional equipment was found to be the following:

$$E_{conv} = \frac{E_{disp}}{f_1 f_1' * c}$$

Then, using this equation, the values of all the parts of the capital costs were calculated:

$$C_{disp} = c * E_{conv} f_i f_i'$$

This was a similar process applied using the running costs and other factors, and utilized the materials costs of the disposable process for the manufacturing costs.

The following equations summarize the other major economic equations used.

$$Net\ Earn._j = \left(Rev._j - Costs_j - Dep._j\right) * (1 - 0.35)$$

$$Discount.Cash\ Flow_j = Net\ Earn._j + Dep._j + FCI_j + WC_j$$

$$Present\ Value_j = DCF_j * (1 + r)^{-n+j}$$

$$NPV = \sum_j PV_j$$

In the present value equation, *n* is the year in which the first capital expenditure is made. This results in the value of the initial investment being valued in dollars of that year, rather than presented at a higher value than what is invested at that time before the plant begins operation. This reduces the value of future earnings based on the present value calculation.

Appendix F: MSDS and SDS Analyses in Brief

The following section is a brief analysis of the chemicals used in the process that have the potential to be problematic or are unique. This is a supplementary section to the Safety, Health, and Environmental Considerations in section 9.0 of the report.

EXCELL 420-
It is a non-hazardous substance. It has HMIS and NFPA ratings of 0 in every category, making it safe for use in the process. It contains L-glutamine, and no other properties are reported in the MSDS [53].

Sodium Bicarbonate-
It slightly hazardous if it comes into contact with skin, eyes, or is ingested. It acts as an irritant. It is not flammable, carcinogenic, or teratogenic. If heated too much, it will emit acrid and irritating smoke. Use safety glasses, lab coat, and dust respirator when using. No exposure limits available [54].

Hydrochloric Acid-
Hydrochloric acid is primarily an irritant if ingested or if a person is exposed. Severe over exposure to this chemical can result in death, if not properly treated. Hydrochloric acid is reactive with certain chemicals, though, it is non-flammable. Wear gloves, eye protection, and safety coats when working with it. If spilled, it can be easily neutralized with a diluted solution of sodium carbonate. If a large spill occurs, it can be contained with dry earth or sand, use water to prevent vapors, and containment is necessary. The PEL is 5ppm or $7mg/m^3$. It is highly reactive with metals and is extremely corrosive [55].

Isopropanol-
It is a slight irritant if it comes into contact with skin. It is toxic to the liver, kidneys, and central nervous system if exposed. UFL 2%. LFL 12.7%. FP 11.667°C. Isopropanol is highly flammable. If it catches fire, either a dry chemical or foam should be used to contain the blaze. Goggles, lab coat, and vapor respirator should be worn at all times with gloves. The 200 STEL is 400ppm [56].

Sodium Phosphate, Dibasic-
Mild skin irritant if exposed. It is not flammable. If it is spilled, it can be moved or shoveled into the nearest waste disposal container. It is only a slight irritant to skin or if ingested [57].

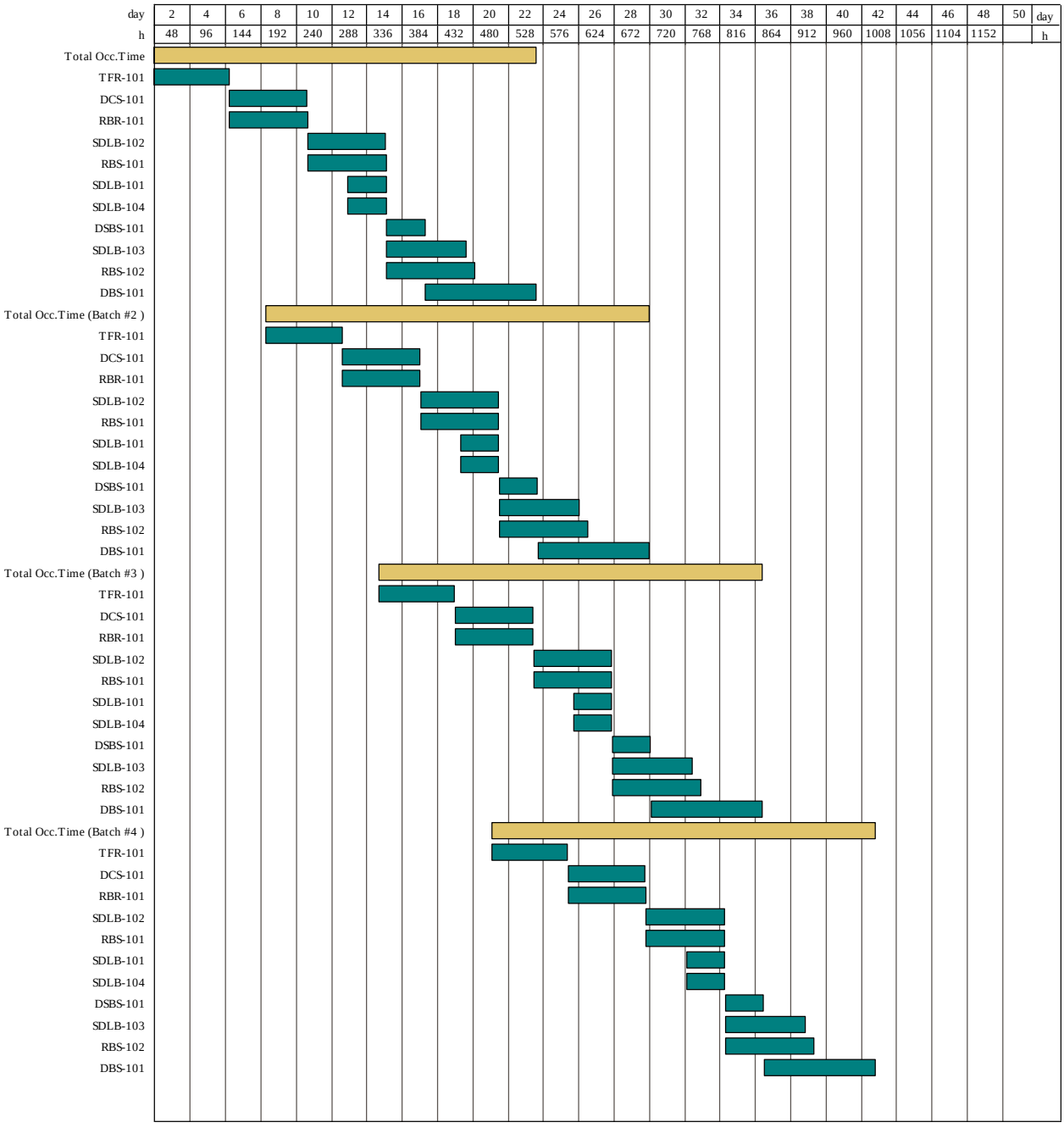
Sodium Phosphate, Monobasic-
It is a slight irritant to the skin or if ingested/inhaled. It is not classified as a hazardous material otherwise. PPE includes splash goggles, dust respirator, gloves, boots, and a full suit [58].

Sodium Hydroxide-
A caustic base that is corrosive to skin, eyes, and dangerous if inhaled or ingested. Damage is based on length of contact, and thus, should be cleaned away/removed as soon as possible. The substance is non-flammable, but is highly reactive with metal materials. It can react with certain chemicals to form explosive components. The TLV is $2mg/m^3$ and the PEL is $2mg/m^3$. Those

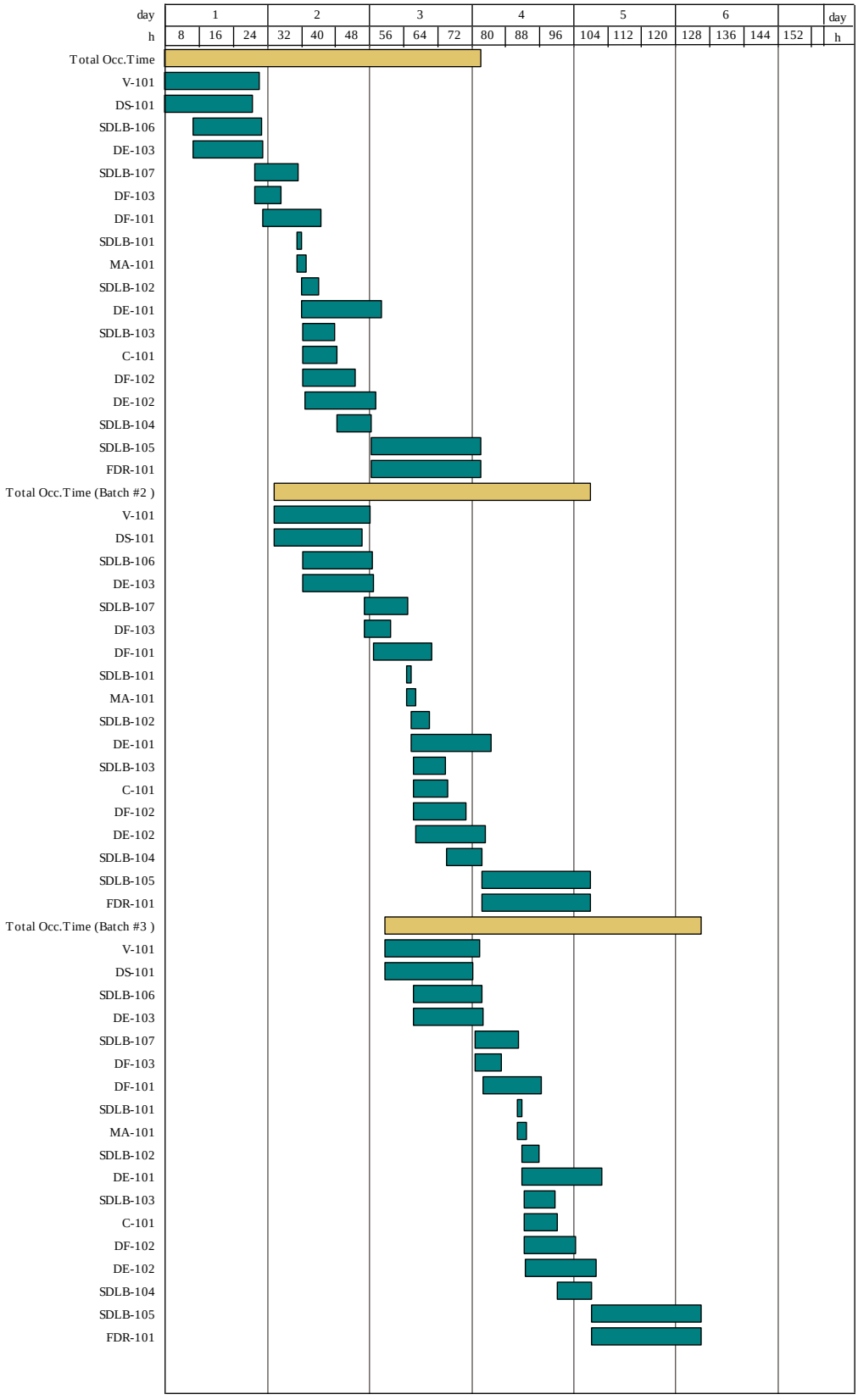
working with it should wear vapor and dust respirators, goggles, boots, gloves, and a synthetic apron. Do not wash away with water and prevent entry into sewers. Contain if possible and dilute with acetic acid solution.

Appendix G: Gantt Charts from SuperPro

G-1: Gantt Chart of the Upstream Process for 1 Section (SuperPro Designer v8.5)



G-2: Gantt Chart of the Downstream Process for 1 Section (SuperPro Designer v8.5)



Appendix H: WHO Summary Protocol [12]

Summary protocol for influenza vaccine (inactivated) (master/working seed lot Type A or Type B)

The model summary protocol that follows is provided as general guidance to manufacturers. It is not intended to constrain them in the presentation of data relevant to the complete review of the quality control tests performed on the vaccine. It is important to note that satisfactory test results do not necessarily imply that the vaccine is safe and effective, since many other factors must be taken into account, including the characteristics of the manufacturing facility.

Name and address of manufacturer

Laboratory reference no. of lot

Date when the processing was completed

Information on manufacture

Virus used to inoculate eggs or cells for the manufacture of the lot:
(a) strain and substrain
(b) passage level
(c) source and reference no.
(d) remarks

Results of sterility test

Results of tests for extraneous agents

Results of tests on adjuvant (if any)

Conditions of storage

Monovalent virus pool Type A or Type B

Name and address of manufacturer

Laboratory reference no. of virus pool

Virus used to inoculate eggs or cells.
(a) master seed strain and source
(b) passage level of master seed
(c) working seed lot, reference no. and source

Date of inoculation

Date of harvesting allantoic or amniotic fluids or cell culture fluids

Storage conditions before inactivation

Date of inactivation

Time of inactivation

Method of inactivation

Concentration of inactivating agent

Storage conditions after inactivation

Concentration/purification procedure

Antibiotics used during preparation, if any

Identification of adjuvant added, if any

Tests on monovalent pool

Test for absence of viable influenza virus

No. of eggs or cell culture vessels inoculated

Incubation time and temperature

Date of test

Results

Determination of haemagglutinin content

Method

Date of determination

Results

Tests for presence of neuraminidase (if performed)

Method

Date of test

Results

Virus disruption (for split vaccine)

Method

Date

Results

Purity (for subunit vaccine)

Method

Date

Results

Purity (for cell-derived vaccine)

Method

Date

Results

Identity tests

Method

Date of test

Results

Test for extraneous agents (if performed)

Method

Date

Results

Final bulk

Name and address of manufacturer

Identification of final bulk

Identification of monovalent virus pool used to prepare final bulk

Date of manufacture

Control of final bulk

Preservative(s) added and concentration

Any other substances added and concentration

Determination of haemagglutinin content

Method

Date of determination

Results

Sterility

Date of test

Results

Total protein content

Method

Date of test

Results

Ovalbumin content (egg-derived vaccines)

Method

Date of test

Results

Test for residual DNA (if performed)

Method

Date

Results

Test for adjuvant (if performed)

Method

Date

Results

Tests for chemicals used

Date of tests

Results

Final lot

Identity test

Method

Date of test

Results

Sterility

Method

Date of test

Results

Determination of haemagglutinin content

Method

Date of determination

Results

Innocuity (if performed)

No. and species of animals

Doses injected

Period of observation

Date of test

Results

Endotoxin content

Method

Date of test

Results

Inspection of final container

Results

Other tests

Additional comments (if any)
A sample of a completed final container label and package insert should be attached.

Certification by producer

Name of head of production of the final vaccine

Certification by head of the quality assurance department taking overall responsibility for production and control of the final vaccine:

I certify that lot no . . . of influenza vaccine (inactivated), whose number appears on the label of the final container, meets all national requirements and satisfies Part A of the Requirements for Biological Substances No. 17, revised 1990.

Signature: _____
Name (typed): _____
Date: _____

Certification by the national controller

If the vaccine is to be exported, provide a copy of the certificate from the national regulatory authority as described in section B.2, a label of a final container, and a leaflet of instructions to users.

Appendix I: Model Certificate from the WHO [12]

Model certificate for the release of influenza vaccine (inactivated)

The following lots of influenza vaccine (inactivated) produced by _____ in _____ whose numbers appear on the labels of the final containers, meet all national requirements, Part A of the Recommendations for Influenza Vaccine (Inactivated) (revised 2003) and the recommendations for good manufacturing practice and quality assurance for biological products.

Lot Number	Date of Last Potency Test by Manufacturer	Expiry Lot Number

As a minimum, this certificate is based on examination of the manufacturing protocol.

The number of this certificate is: _____

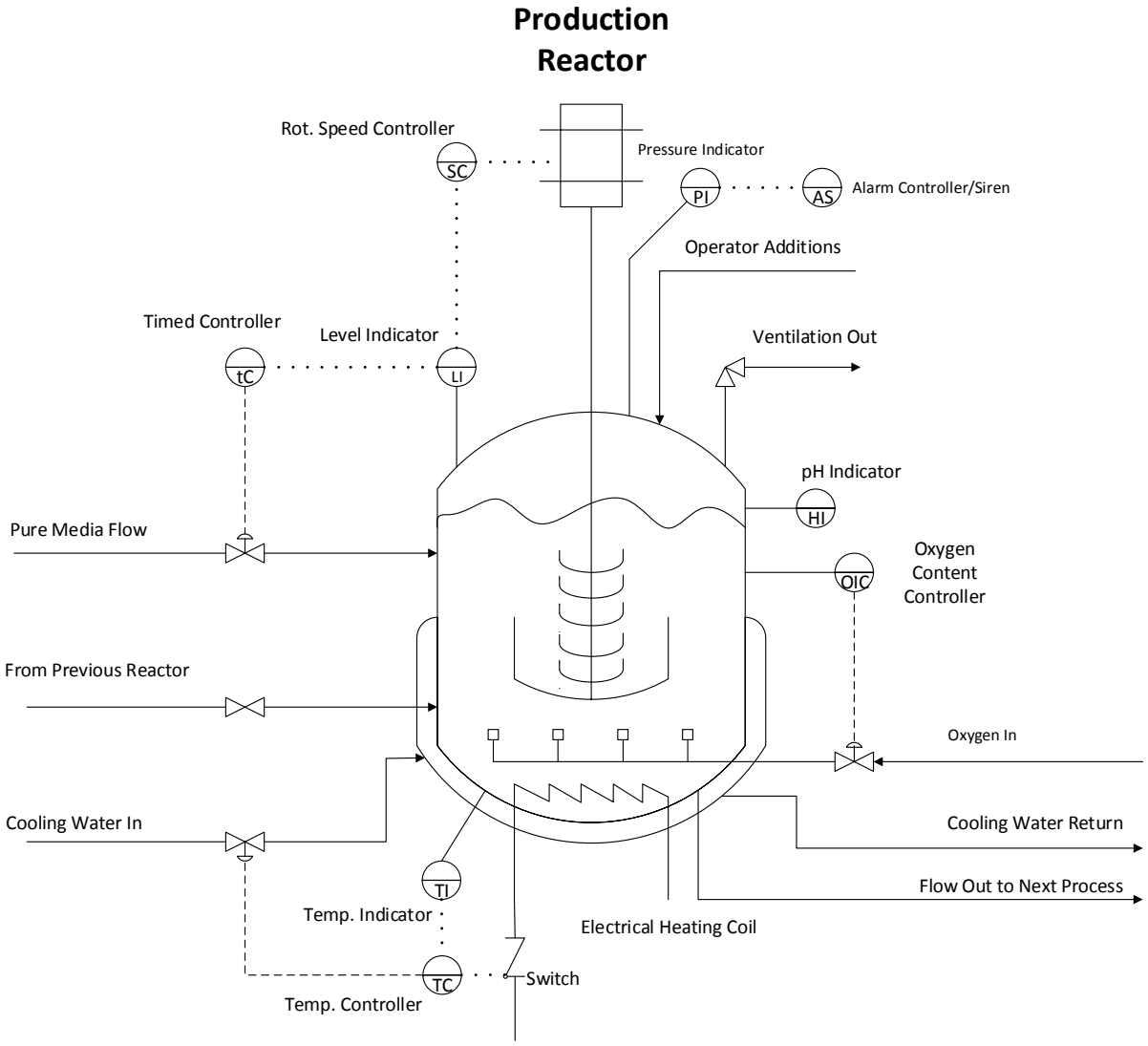
The Director of the National Control Laboratory (or Authority as appropriate):

Name (typed): _____

Signature: _____

Date: _____

Appendix J: Sample Piping and Instrumentation Diagram



The level indicating controller controls both the rotational mixer speed and the flow into the tank. The rotational speed is automatically set by an operator, but if at any given time the surface of the liquid becomes too turbulent for the indicator to read, the rotational speed controller will slow the motor down.

The temperature controller will move cooling water or run the heating coil, based on the set point and the indicated temperature. If it is too hot, the switch is flipped open and the cooling water valve is opened, turning off the electricity and the water on. If it is too cold, the switch is closed, and the valve closed, leading to the heat being turned on and the cooling water turned off.

The pH indicator merely outputs the pH to any nearby operator. If at any given time the pH becomes too high or too low, the operator can add standard solutions into the reactor to try to increase or decrease the pH. However, the literature indicates this should not be an issue.

The vent allows for exiting gasses to be removed. It is assumed that the bag/reactor is slightly above atmospheric pressure due to the vent, i.e., no pressure builds up, just flow through. However, if pressure builds up (i.e., the valve gets shut), then an indicator will monitor the pressure. At a certain psig, the alarm controller will set of a siren, alerting area operators that the reactor has a pressure build up in it that needs to be immediately rectified.

There will be two options for the alarm unit to use, which is not indicated by the diagram. The first is a low pressure alarm. This means that pressure has built up and needs to be rectified, but catastrophic failure is not imminent if the issue is rectified. The second alarm is a high pressure alarm. This alarm alerts operators to immediately clear the area and try to quickly rectify the issue. Should this alarm go off, it means catastrophic failure is immediately imminent if the situation is not rectified.

Appendix K: Sample HAZOP Analysis

Equipment Reference and Operating Conditions	Deviations	Causes?	Consequences	Additional Consequences	Process Indicators
Production Reactor	**Level**				
	Less	Media Runs Dry	Pump Cavitation	Pump Damage	LI, tC
		Previous Reactor not Empty	Product Loss	Potential Contamination	
		Media Flow Valve Open/Broken	Releases	Contamination	tC
		Previous Reactor Valve Open/Broken	Releases	Contamination, Exposure	
		Tank Rupture	Releases	Contamination, Exposure	
	More	Too much Media Loaded	Overfill	Rupture	LI
		Backflow from Downstream	Overfill	Rupture	LI
	None	Same as Less			
	Pressure				
	Less	Too low flow from oxygen	Product Loss	Reactor Implosion	DIC, PI
		Too little media flow in	See Level Less		tC, LI, PI
		Line Rupture	Releases	Contamination	DIC, TC, tc, LI, PI
		Tank Rupture	Releases	Contamination, Exposure	PI, LI
	More	Too much from oxygen	Over Oxygenation	Product Loss, Ruptures	DIC, PI, AS
		Media flow too high	Overfill	Rupture	tC, PI, AS
		Ventilation Valve Broken	Releases	Contamination	PI, AS
	None	Rupture	Releases	Contamination, Exposure	PI, LI, AS
	Temperature				
	Less	Oxygen Valve Stuck Open	Product Loss	Rupture	DIC, TI
		Electrical Switch Broken	Product Loss		TI, TC
		Cooling Water Valve Stuck Open	Product Loss	Rupture of Cooling Jacket	TI, TC
	More	Cooling Water Valve Stuck Closed	Product Loss	Tank Melts/Rupture	TI, TC
		Electrical Switch Broken	Product Loss	Tank Melts/Rupture	TI, TC
	pH				
	Less	Operator added too much acid	Product Loss	Tank Rupture	HI
		Too much oxygen	Product Loss	Tank Rupture	HI
		Tank Rupture	Releases	Contamination, Exposure	HI
	More	Operator added too much base	Product Loss	Tank Rupture	HI
		Reaction proceeded to long	Product Loss		HI
		Tank Rupture	Releases	Contamination, Exposure	HI
	Oxygenation				
	Less	Oxygen Valve Stuck Closed	Product Loss		DIC
		Oxygen Line Rupture	Releases	Contamination	DIC
	More	Oxygen Valve Stuck Open	Over Pressurization	Rupture, Releases	DIC

	Mixer Speed				
	Less	Set point too low	Product Loss		SC
		Controller broken	Product Loss	Motor Damage	SC
		Motor broken	Product Loss	Motor Damage	SC
	More	Set point too high	Motor Damage	Motor Destruction	SC
		Controller broken	Motor Damage	Motor Destruction	SC
		Level indicator broken	Product Loss	Motor Destruction	LI, SC
		Disruption to reactor	Rupture	Contamination, Exposure	SC