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From: Ghazal Erfani, Ciera Lowe, and Joshua Mayourian
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To: Diamond Venture Capital

Date: May 19, 2014

Dear Client,

On the behalf of C Squared Consulting, we present to you, “Manufacturing the Next Generation of Vaccines: Non-Egg Based Platform for Influenza Vaccine”, a potential investment opportunity for your firm.

We created a novel highly profitable platform to manufacture approximately 50 million doses of trivalent influenza vaccine in safe and environmentally conscious manner. Using newly developed biotechnology, SF9 insect cells will be infected with recombinant Baculovirus that expresses influenza strain using a non-traditional disposable approach.

For a plant life of 8 years, a dosage price as low as \$0.44/dose is required to obtain a beneficial investment rate of return of 25% and total profit of \$60 million. Current egg-based versions of the flu vaccine require a dosage price as high as \$0.64/dose to obtain a similar investment rate of return and total profit. With our design at a selling price of \$0.64/dose, it is possible to achieve an investment rate of return as large as 42%, and a total profit of \$120 million. Therefore, we recommend going forward with this plant design.

If you have any questions on these matters, please contact Joshua Mayourian at 555-555-5555.

Sincerely,

Ghazal Erfani

Ciera Lowe

Joshua Mayourian

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MANUFACTURING THE NEXT GENERATION OF
VACCINES: NON-EGG BASED PLATFORM FOR
INFLUENZA VACCINE

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May 19, 2014

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Abstract

The influenza virus causes approximately 17,000 to 51,000 deaths a year in the U.S. alone, and could potentially cause over a million deaths globally. This pandemic therefore is a global health and economic concern, making it of interest to develop a vaccine. Specifically, a new trivalent flu vaccine will be developed using SF9 insect cells for influenza A, B-hemagglutinin, and B-neuraminidase. We seek to produce 50 million doses of flu vaccine, which is approximately one-third of the total flu vaccine distributed during the 2013-2014 influenza season in the United States. To develop this trivalent flu vaccine, a non-traditional disposable approach will be taken, with the following steps: 1) preparing media and buffer; 2) expanding cells by a seed train where cells are multiplied; 3) producing vaccine by inoculating the cells with the recombinant Baculovirus; 4) recovering the contents and products via centrifugation and filtration; 5) inactivating the virus; 6) capturing and purifying the vaccine of interest; and 7) concentrating and stabilizing the material for shipping. Accounting for the cost of each of these steps, the cost of good sold is \$0.08 per dose. For a plant life of 8 years, a dosage price as low as \$0.44/dose is required to obtain a rewarding investment rate of return of 25% and total profit of \$60 million. Current egg-based versions of the flu vaccine require a dosage price as high as \$0.64/dose to obtain a similar investment rate of return and total profit. With our design at a selling price of \$0.64/dose, it is possible to achieve an investment rate of return as large as 42%, and a total profit of \$120 million.

Introduction

Influenza virus is an infectious disease that causes approximately 17,000 to 51,000 deaths a year in the U.S. alone, and could potentially cause over a million deaths globally [1]. This acute viral infection attacks the respiratory system and spreads easily from person to person. The influenza pandemic can take an economic toll through lost workforce productivity as well as strain health services [2], making influenza vaccines of interest. Therefore, we seek to produce influenza vaccine at a cheap cost and an environmentally conscious manner.

1.1 Product of Interest: Trivalent Influenza Vaccine

The influenza vaccine generates antibodies within the human body to protect against the influenza virus [3]. Each year, the World Health Organization and its partners predict what strains will be most impactful in the following influenza seasons, and recommend the appropriate virus strain vaccinations accordingly [4]. For the 2013-2014 flu season, the following strains (two type-A strains, and one type-B strain) were recommended, making a trivalent influenza vaccine of interest (Table 1.1).

TABLE 1.1: Strains Recommended for 2013-2014 Flu Season

Influenza Strain	Lineage	Reference
A: H1N1	A/California/7/2009	[5]
A: H3N2	A/Victoria/361/2011	[6]
B	B/Massachusetts/2/2012	[7]

1.2 Motivation for Entering Market

The number of vaccines administered each year has steadily increased over the past few years (Figure 1.1) [8, 9], presenting the opportunity for another biotechnology company to enter the field.

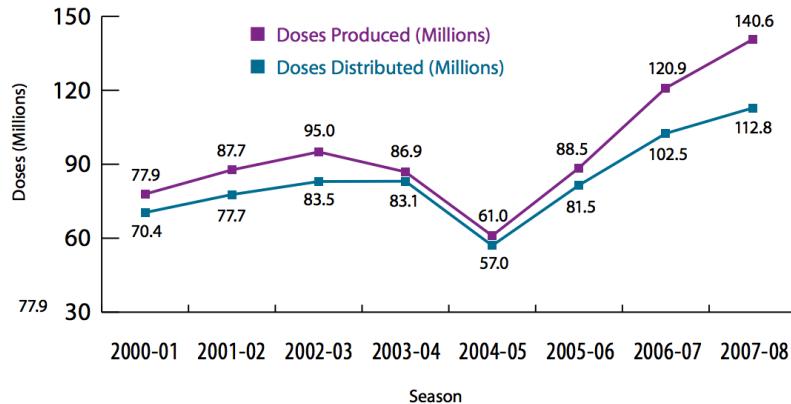


FIGURE 1.1: The number of vaccines administered each year has steadily increased over the past few years [8, 9].

Each year, approximately 135 million doses are administered in the United States, including 50 million, 40 million, and 35 million by Sanofi Pasteur, Novartis, and Galxo-SmithKline, respectively (Figure 1.2) [8].

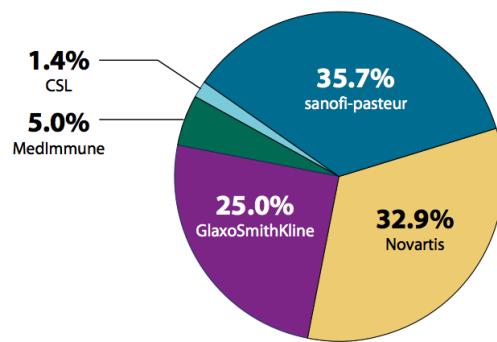


FIGURE 1.2: Each year, approximately 135 million doses are administered, including 50 million, 40 million, and 35 million by Sanofi Pasteur, Novartis, and GalxoSmithKline, respectively [8].

As a current world leading vaccine company, we believe we can sell 50 million trivalent vaccine doses per year, as long as we maintain reduced prices. Currently, our main

competitors sell each dose to distributors at \$0.64/dose to achieve an investment rate of return of 25% (see Section 12 for details on how this price was determined) [10]. Therefore, to compete effectively in this market, we seek to sell 50 million trivalent vaccine dose per year at under \$0.64/dose [10]. With new biotechnology available, we are confident this is possible.

1.3 State-of-the-Art Methods of Manufacturing Trivalent Influenza Vaccine

Various types of hosting environments and manufacturing facilities can be used within biotechnology industry to mass produce this trivalent influenza vaccine, as discussed in the following section. These techniques motivated our non-traditional approach of using disposable equipment, and using insect cells as the hosting environment for vaccine production.

1.3.1 Hosting Environment for Vaccine Production

Historically, trivalent influenza vaccines have been produced in chicken or duck eggs. This method of vaccine production is well-documented and has been approved worldwide [11, 12]. It has allowed for the quick development of new vaccines each year, meeting the time and quantity constraints set by this seasonal virus. In egg-based influenza vaccine production, the viruses are injected into the chicken or duck egg, and the strains of interest are inactivated, separated, and packaged into individual vaccine doses [11, 12].

However, there are many risks that go along with this egg-based platform [12]. Egg supply is not reliable, and may become unavailable for various reasons at any point. Furthermore, individuals allergic to eggs are advised to avoid egg-based influenza vaccine [9]. Therefore, other improved methods have been proposed.

A newer non-egg based method of vaccine production has recently gained approval by the Food and Drug Administration [13, 14]. This method uses mammalian or insect cells as the host for the growth of influenza virus. This technique is more reliable and flexible than the traditional method. It also allows for a faster start-up of the manufacturing process in the event of a pandemic. Using this cell-based method ensures an adequate supply of cells readily available for vaccine production. Furthermore, it is a more efficient and cost-effective method than the traditional egg-based method of production [13, 14].

In our design, we selected insect cells as hosts to grow the vaccine (see Section 1.4 for more details on why we selected this method).

1.3.2 Manufacturing Facilities

Producing an influenza vaccine also requires designing a good manufacturing practice (GMP) commercial manufacturing facility. Typically, biotechnology companies choose between three types of facilities:

1. A traditional team-in-place and clean-in-place production facility (either at the company site, or at a contract manufacturer).
2. A mix between the traditional facility described above with the non-traditional disposable bioreactor and vessel approach (either at the company site, or at a contract manufacturer).
3. A complete non-traditional facility, using disposable equipment throughout the process (either at the company site, or at a contract manufacturer).

In our design, we selected a complete non-traditional facility that uses disposable equipment. Furthermore, we will use a contract manufacturing organization (CMO) for the production of our vaccine (see Section 1.4 and 1.5 for more details on why we selected this method).

1.4 Method Selected for Producing Trivalent Influenza Vaccine Production

As part of a world leading vaccine company initiative, we are creating a new platform within our company to manufacture influenza vaccines using cell culture. Once proof of concept has been obtained, this platform will be considered for current and future products beyond influenza. The trivalent vaccine made using this alternative non-egg based method will have the same potency as the egg-based process and follow the World Health Organization's recommendations for the North American market each year. Overall, our process and design will embrace the latest technological advances in the vaccine industry.

We will use insect cell cultures in disposable technology for its simplicity and security, speed of production, high yield, and low yield variation. Specifically, we will use SF9 insect cells to express the trivalent flu vaccine, with the corresponding Sf-900 III SFM

TABLE 1.2: Comparison of Vaccine Production Methodologies

Attribute	Egg-based Culture	Mammalian Cell Culture	Insect Cell Culture in Disposable Technology
Simplicity and Security		✓	✓
Speed of Production		✓	✓
High Yield	✓		✓
Low Yield Variation			✓
Low Facility Cost			✓
Fast Construction and Validation Time			✓
Ease of scale-up		✓	✓

medium for the insect cells to grow. This medium has a low hydrolysate concentration (which reduces cell culture variability), and is not of animal origin.

The comparison between insect cell cultures in disposable technology and other methods (mammalian cell culture and egg-based) shows that this non-traditional method is the most beneficial (Table 1.2) [15]. This disposable approach was also selected for its significant economic benefits, low capital cost, fast construction, ease of scale-up, and fast investment payback demonstrated for distinguished biotechnology companies. Finally, this manufacturing approach results in a completely closed system, which avoids leaks and allows for a much safer environment.

1.5 Production Level and Plant Location

The design and construction of this manufacturing facility will be complete in coordination with a contract manufacturing organization (CMO). The CMO will provide comprehensive services throughout the manufacturing processes such as formulation development, stability studies, method development, and scale-up. Outsourcing to a CMO also allows us to expand technical resources without increased overhead. Additionally,

we will be able to manage internal resources and costs by focusing on core competencies and high-value projects, while reducing infrastructure and staff. A summary of the advantages of using a biopharmaceutical contract manufacturing organization is shown in Figure 1.3 [16].

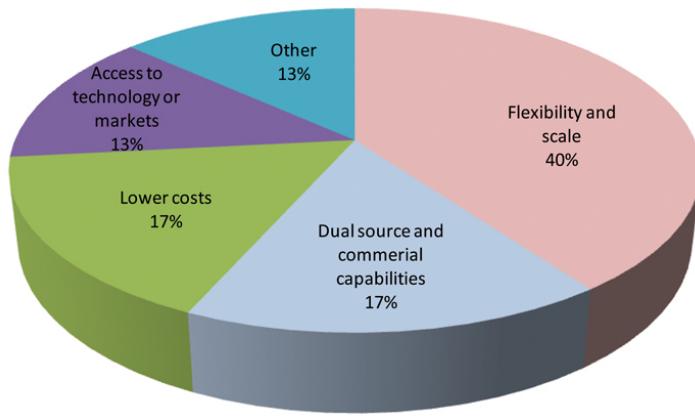


FIGURE 1.3: Advantages of using a CMO include access to technology and markets, lower costs, multiple sources and commercial capabilities, and flexibility and scale [16].

Working with a CMO also entails giving up direct control of the project in regards to cost and scheduling [17]. The CMO we will be working with is Baxter BioPharma Solutions, as it has been named the “Best Contract Manufacturing Organization” for three consecutive years at Vaccine Industry Excellence Awards [18].

1.5.1 Plant Location

Since most of the work done by Baxter is located in Round Lake, Illinois, this location will be used for our new manufacturing facility [18]. Furthermore, we selected this location for its cheap cost, acceptance of biotechnology companies in the region, and weather. A 100,000 square foot facility will be required for our production plant and offices (for details of this facility, see Section 10.3).

1.6 Potential Environmental Issues and Safety Hazards

Vaccine production requires numerous safety, health, and environmental considerations. The safety and health considerations include: 1) maintaining a sterile environment throughout our process; 2) controlling and isolating activated virus; 3) properly storing inactivated virus; 4) avoiding risk of contamination and cross-contamination throughout the

process; 5) maintaining a safe environment for all employees; and 6) meeting good manufacturing practices. The environmental considerations include: 1) minimizing the waste produced in this process (i.e. having an energy efficient process), and 2) properly disposing of the solid, liquid, and gas wastes. Each of these safety, health, and environmental concerns are resolved in detail in Section 9.

Process Flow Diagram and Material Balances

2.1 Process Flow Diagram

To develop this trivalent flu vaccine, a disposable approach will be taken, with the following steps: 1) preparing media and buffer; 2) expanding cells by a seed train where cells are passaged into larger volumes; 3) producing vaccine by entering the cells into production bioreactors; 4) recovering the contents and products via centrifugation and filtration; 5) inactivating the virus; 6) capturing and purifying the vaccine of interest; and 7) concentrating and stabilizing the material for shipping. A block flow diagram of this general process is shown below.

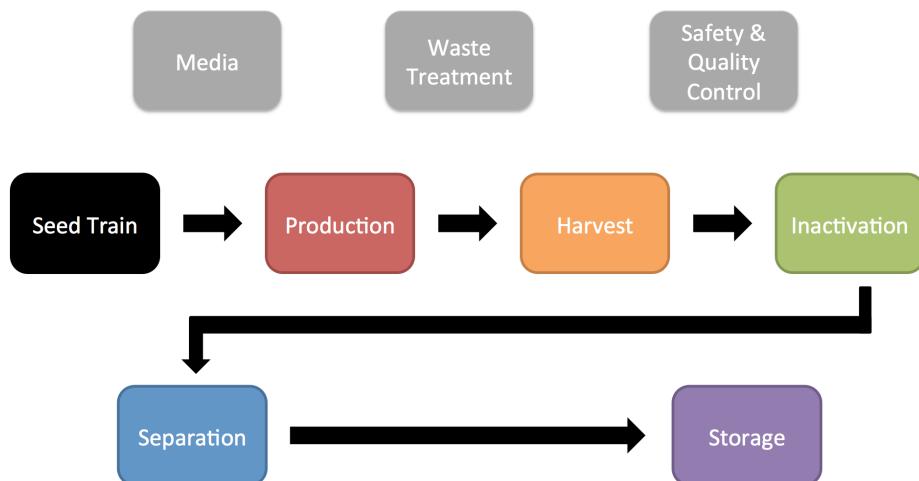


FIGURE 2.1: Block Flow Diagram of the Vaccine Production Process. This process includes preparing media and buffers, a seed train, producing the vaccine, harvesting the products, inactivating the virus, separating the products, and storing the vaccine.

A more detailed process flow diagram of this process is shown below. The specifications indicated within the process flow diagram are for each batch process. In total, there are 21 batches per year.

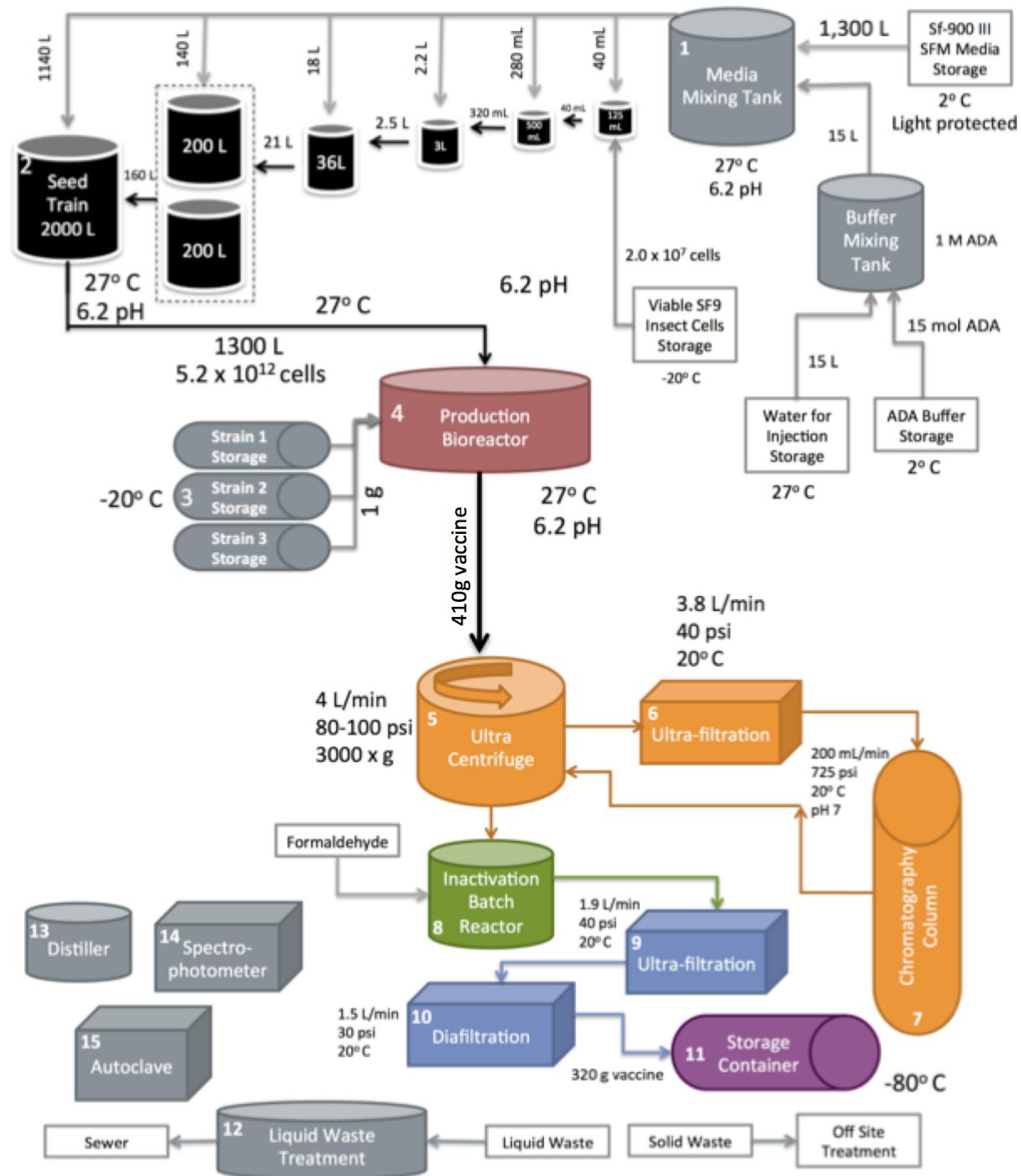


FIGURE 2.2: Process Flow Diagram of the Vaccine Production Process. This process includes preparing media and buffers, a seed train, producing the vaccine, harvesting the products, inactivating the virus, separating the products, and storing the vaccine.

To find the reactants and products for each step in the process, a material balance was made. This material balance is shown in the following section.

2.2 Material Balance Block

To complete a material balance, the following assumptions were made in the seed train based on literature data:

1. For optimal cell growth, cells should enter each bioreactor at a density of 5×10^5 cells/mL, and exit each bioreactor at a density of 4×10^6 cells/mL (i.e. mid-log phase) [19].
2. To model the cell growth in the seed train, the following exponential relationship is used:

$$P(t) = P_o(2^{t/\tau_o}) \quad (2.1)$$

where $P(t)$ is the population at time t , P_o is the initial population (5×10^5 cells/mL), t is the time in the reactor, and τ_o is the doubling time. Using this relationship, τ_o was fit to data found for population growth over time (Figure 2.3) using Sf-900 III SFM media, and calculated as 24 hours [19].

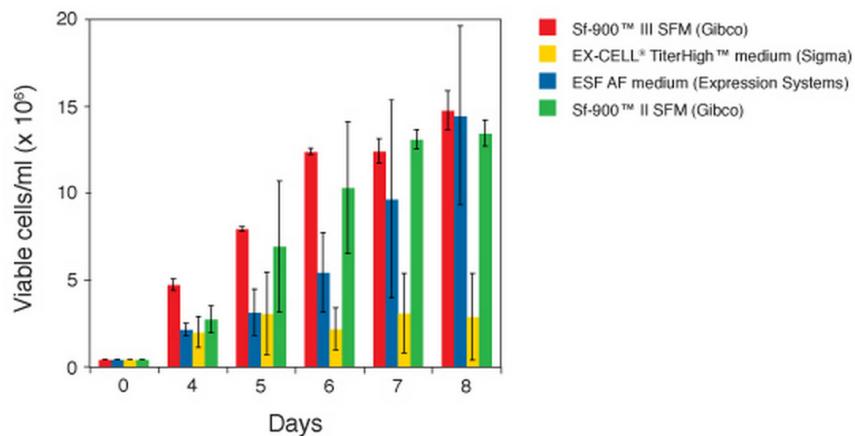


FIGURE 2.3: An exponential growth relationship was fit to this empirical data to model cell growth in Sf-900 III SFM media [19].

3. There is a total efficiency of 98% for transferring from bioreactor to bioreactor in the seed train stage.
4. The buffer should have a molarity of approximately 10 mM in media [20].

5. For proper aeration, each seed train bioreactor is filled to 60% of maximum capacity [21].
6. It is not necessary to change medium for each reactor batch, as the addition of medium is sufficient to replenish cell nutrients [21].

The following assumptions were made in the production bioreactor based on literature data:

1. For proper aeration, each production bioreactor is filled to 70% of maximum capacity [21].
2. No cell growth occurs following virus incorporation [22].
3. The virus grows at $77 \mu\text{g}/10^6 \text{ cells}$ [23].
4. There is a total efficiency of 99% for this process.

Finally, the following assumptions on virus separation efficiency were made in the downstream processes based on literature data:

TABLE 2.1: Separation Efficiency

Unit	Efficiency	Reference
Ultra-centrifuge	0.98	[24]
Ultra-filtration	0.99	[25]
Chromatography	0.86	[26]
Diafiltration	0.99	[27]

We seek to make 50 million trivalent influenza doses, where each dose contains a total of $135 \mu\text{g}$ of vaccine (i.e. $45 \mu\text{g}$ of each strain). Therefore, the total amount of vaccine that must be made over one season is 6,750 g. Since we plan on running 21 batches (see Chapters 4 and 10 for justification as to why), a total of 325 g vaccine must be produced per batch. The resulting buffer/media preparation, seed train, production bioreactor, and downstream material balances are shown in Tables 2.2, 2.3, 2.4, and 2.5, respectively. Detailed calculations for these material balances can be seen in Appendix A.

TABLE 2.2: Media/Buffer Material Balance Per Batch

Material of Interest	Quantity
Water for Injection	15 L
ADA Buffer	15 mol
Sf-900 III Media	1,300 L

TABLE 2.3: Seed Train Material Balance

Reactor	Material of Interest	Quantity
125 mL Flask	Media Required From Media Mixing Tank Cells Entering Cells Exiting	40 mL 2.0×10^7 cells 1.6×10^8 cells
500 mL Flask	Media Required From Media Mixing Tank Cells Entering Cells Exiting	280 mL 1.6×10^8 cells 1.3×10^9 cells
3 L Flask	Media Required From Media Mixing Tank Cells Entering Cells Exiting	2.2 L 1.3×10^9 cells 1.0×10^{10} cells
36 L Bioreactor	Media Required From Media Mixing Tank Cells Entering Cells Exiting	18 L 1.0×10^{10} cells 8.3×10^{10} cells
Each 200 L Bioreactor	Media Required From Media Mixing Tank Cells Entering Cells Exiting	71 L 4.2×10^{10} cells 3.3×10^{11} cells
2000 L Bioreactor	Media Required From Media Mixing Tank Cells Entering Cells Exiting 2000 L	1140 L 6.7×10^{11} cells 5.3×10^{12} cells

As a result, 1,300 L of media is required per batch to grow 5.3×10^{12} cells. These cells within the 1,300 L of media are then transferred into the production bioreactor. The material balance for the production bioreactor is shown in Table 2.4

TABLE 2.4: Production Bioreactor Material Balance

Material of Interest	Quantity
Cells Entering	5.3×10^{12} cells
Cells Exiting	N/A
Virus Entering	0 g
Virus Exiting	410 g
Media Entering	1,300 L
Media Exiting	1,300 L

Finally, the downstream material balances are shown in Table 2.5. For details on our calculations on the material balances, see Appendix A.

TABLE 2.5: Downstream Material Balance

Unit	Mass of Vaccine Exiting Unit (g)
Ultra-centrifuge	400
Ultra-filtration	396
Chromatography	336
Ultra-centrifuge	328
Inactivation Reactor	328
Ultra-filtration	324
Diafiltration	321

Process Description

To produce 50 million doses of trivalent influenza vaccine, a disposable approach will be taken, with the following steps:

1. Media and buffer is prepared for the seed train and the production bioreactor.
2. A small volume of viable insect cells are multiplied into a larger cell volumes via a seed train. This step will also avoid the wash out of insect cells with the appropriate precautions.
3. The influenza virus will be produced in the production bioreactor, where the cells are inoculated with a single strain of the virus.
4. The media is separated from the virus in the harvest section.
5. The virus is inactivated in the inactivation section, where the virus still maintains its functionality.
6. The virus is removed from media and subsequently stabilized in the separation section.
7. The stabilized protein is stored in the storage section, until it is ready for shipment.

An overview of the process can be found in Figure 2.2. Each of these steps will be discussed in detail below.

3.1 Preparation of Media and Buffer

First, the media and buffer is prepared for the seed train and production bioreactors. This step is labeled as “1” in Figure 2.2. For each batch, 1,300 L of Sf-900 III SFM media is introduced into the media tank. Furthermore, 15 L of 1 M N-(2-Acetamido)iminodiacetic

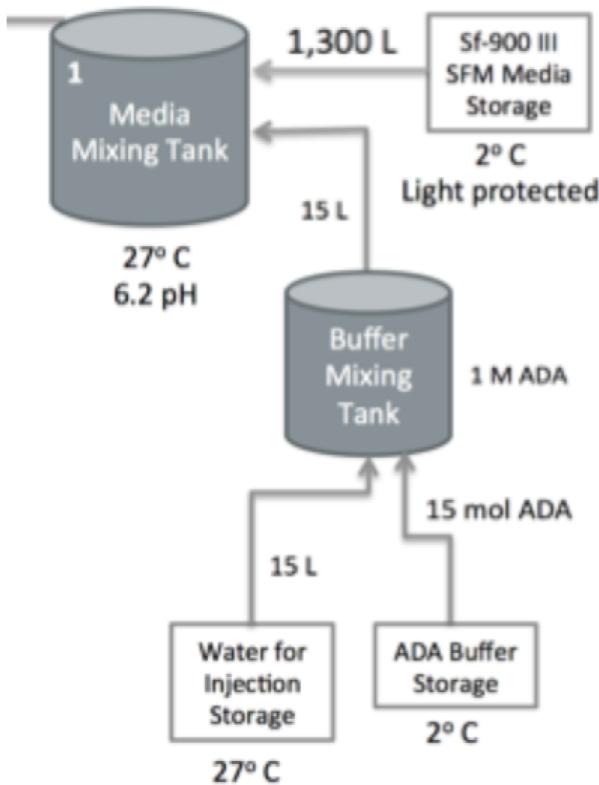


FIGURE 3.1: For each batch, 1,300 L of Sf-900 III SFM media is introduced into the media tank. Furthermore, 15 L of 1 M N-(2-Acetamido)iminodiacetic acid (ADA) buffer is introduced into the mixing tank to immobilized pH gradients, resulting in 10 mM of ADA in the media tank.

acid (ADA) buffer is introduced into the mixing tank to immobilized pH gradients, resulting in 10 mM of ADA in the media tank. The ADA buffer and Sf-900 III SFM media is stored at 2°C, while the mixing tanks and water for injection storage are at 27°C. Literature shows that the pH of the resulting media is 6.2 [19]. An in-detail view of this step is shown in Figure 3.1. The resulting media is introduced into the seed train step.

3.2 Seed Train

The seed train includes seven flasks and bioreactors: one 125 mL flask, one 500 mL flask, one 3 L flask, one 36 L flask, two 200 L bioreactors, and one 2000 L bioreactor. The seed train step is labeled as “2” in Figure 2.2. The goal of the seed train is for a small volume of viable insect cells are multiplied into a larger cell volumes via a seed train. Therefore, the cells enter each bioreactor at a density of 5×10^5 cells/mL, and exit at a density of 4×10^6 cells/mL.

First, 2.0×10^7 cells are introduced into the 125 mL flask, which is filled with 40 mL of Sf-900 III media. After three days, the cells and media are removed and placed into a 500 mL flask that has 280 mL of Sf-900 III media added to it. This propagation step previously described is repeated for the 3 L and 36 L flasks, but instead, 2.2 L and 18 L of Sf-900 III media are added to each flask, respectively. After three days in the 36 L flask, half of the cells and media are placed into one 200 L bioreactor with 70 L of media in it, and the other half is placed into an identical 200 L bioreactor. After three days, the two compositions are combined and placed into a 2000 L bioreactor, which is filled with 1,140 L of media. As a result, 1300 L of media with 5.2×10^{12} cells is transferred to the production bioreactor. The conditions throughout this process are 27°C and a pH of 6.2. An in-detail view of this step is shown in Figure 3.2.

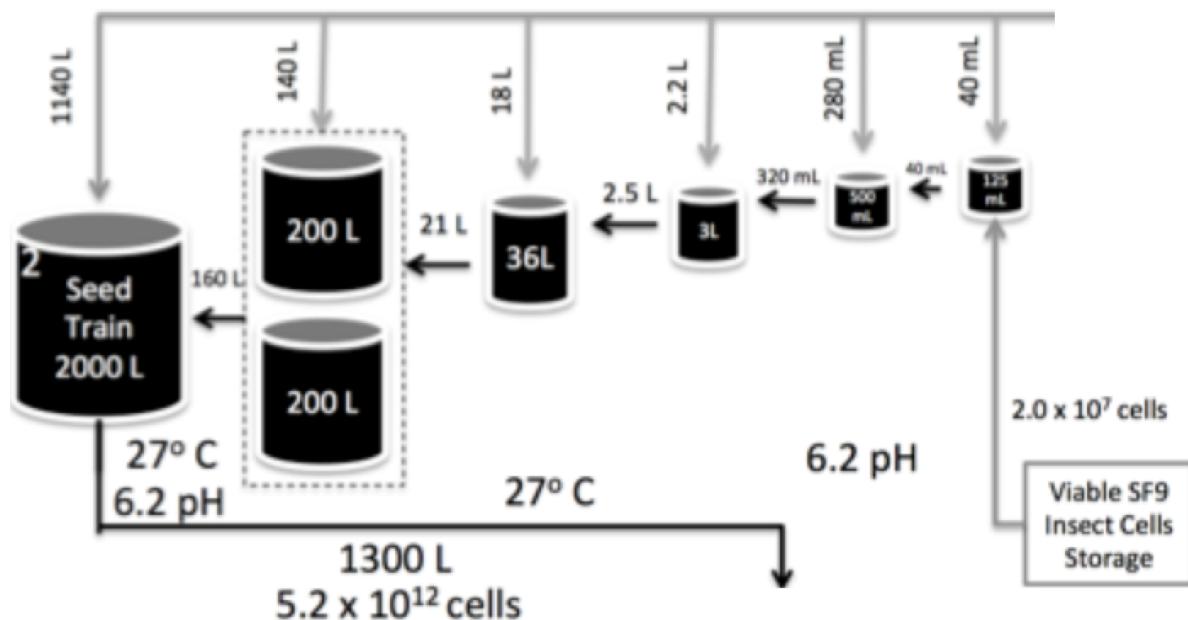


FIGURE 3.2: The seed train includes seven flasks and bioreactors: one 125 mL flask, one 500 mL flask, one 3 L flask, one 36 L flask, two 200 L bioreactors, and one 2000 L bioreactor. The goal of the seed train is for a small volume of viable insect cells to be multiplied into a larger cell volume via a seed train.

3.3 Production

The resulting cells and media are transferred into the production bioreactor, which is labeled as “4” in Figure 2.2. Furthermore, 1 g of a single strain of influenza virus is introduced into the production reactor from the virus storage tanks (labeled as “3” in

Figure 2.2). This allows for the virus to attack the cells and reproduce rapidly. After 108 hours, 410 g of a single strain of influenza virus is produced. Since the virus co-exists with various by-products (i.e. media and dead cells), it is necessary to separate and inactivate the virus. An in-detail view of this step is shown in Figure 3.3.

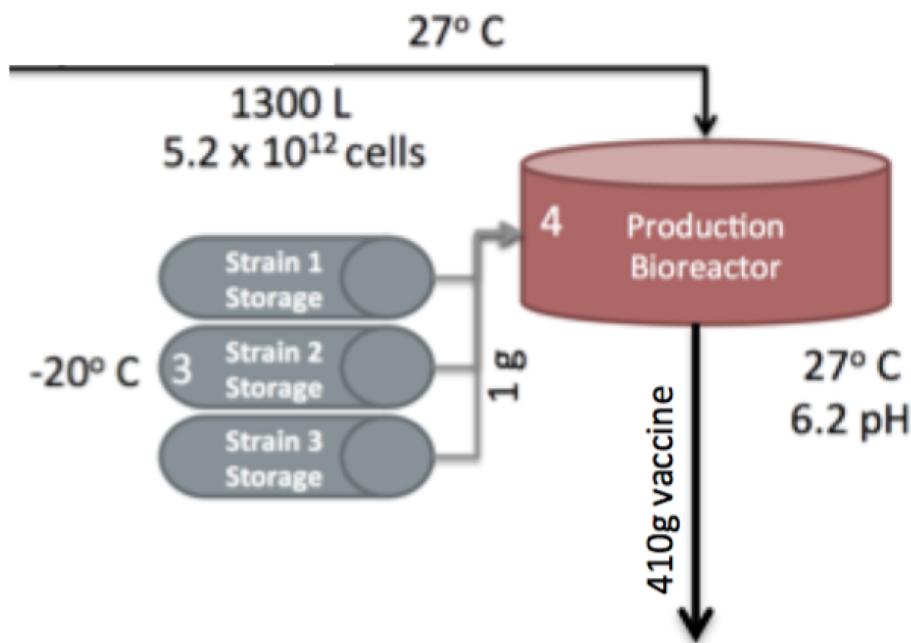


FIGURE 3.3: 1 g of a single strain of influenza virus is introduced into the production reactor from the virus storage tanks, which produces 410 g of a single strain of influenza virus after 108 hours.

3.4 Harvesting

The first and last step in the harvesting section is to feed to media to the ultra centrifuge, which is labeled “5” in Figure 2.2. The first pass through the centrifuge is designed to pellet out a majority the cells without lysing them, and the unit includes a clarification step to remove any cell debris that may be floating on the media’s surface. From here the media is passed through the ultra filtration, labeled “6” in Figure 2.2, in order to remove more of the cells that are still suspended in the media as well as other impurities. The chromatography column, labeled “7” in Figure 2.2, is operated such that during the first pass of media through the column the virus like protein adheres to the packing. Then a sodium chloride solution is used to elute the protein. From here the sodium choloride solution is passed through the ultra centrifuge one additional time to ensure all the cells have been pelleted out. Each of these units are operated at 20°C , and various pressures as

shown in Figure 2.2. The resulting 328 g of vaccine product, and associated by-products, are subsequently transferred to the inactivation section.

3.5 Inactivation

In the inactivation section, formaldehyde is added to the sodium chloride solution to inactivate the virus. The inactivation takes place within a mixing tank at 20°C. This unit is labeled as “8” in Figure 2.2.

3.6 Separation

Following inactivation, another separation step occurs. The resulting inactivated solution is passed through an ultra-filtration module, labeled as “9” in Figure 2.2, and a diafiltration unit, labeled as “10” in Figure 2.2, to filter out the sodium chloride in the solution and any other impurities which may remain. Furthermore, this separation is performed to separate as much of the media as possible for ease of storage. This process is performed at 20°C.

3.7 Storage

Finally, the virus is stored in biocontainers, and placed in a freezer, labeled “11” in Figure 2.2. The virus is stored at -80°C.

3.8 Auxillary Equipment

Auxillary equipment exists throughout this process, as shown in Figure 2.2. For example, the liquid waste treatment unit, labeled “12” in Figure 2.2, is needed to treat the liquid by-products. The quality control and safety testing equipment such as the distiller, spectrophotometer, and autoclave, labeled as “13”, “14”, and “15” in Figure 2.2 respectively, are used to ensure excellence in our production process. The distiller is used to prepare sterile water necessary for testing the product. The spectrophotometer is used to test the concentration of samples of the cells suspended in media, which determines precisely when media should be moved from one reactor to the next. Finally the autoclave is used to sterilze all of the equipment used in the plant.

Energy Balance and Utility Requirements

4.1 Energy Requirements

Typical disposable-approach bioprocesses have negligible energy requirements and energy balances, making it another advantage of this method. Therefore, it will be excluded in this analysis, as all heat requirements are met by electrical utilities. For example, the kill tank, which heats the liquid waste to treat it, only runs on electricity. Furthermore, the virus storage tanks are maintained in industrial liquid nitrogen cryogenic containers. Finally, cold rooms run on air conditioning units, which run on electricity.

4.2 Utility Requirements

Various utilities are required for this plant, including electricity, water, and sewage. Electricity is required for each bioreactor, separation unit, air conditioning units, and the kill tank. Electricity is required for the bioreactors to operate the control systems on pH, carbon dioxide content, and temperature. Electricity is required for each separation unit for supplying the appropriate operating conditions. Air conditioning units require electricity to keep the temperature of the cold room at -20°C. Finally, for the kill tank, electricity is required to heat the liquid waste to the appropriate temperature to make it suitable to be disposed of in the sewers.

Water, the next utility, is required for buffer preparation during the buffer and media preparation stage. Finally, sewage is required to dispose of the liquid waste after it is properly treated in the kill tank. The total electricity, water, and sewage utility requirements are shown in Table 4.1. The pricing for each of these utility requirements are discussed in further detail in Section 11.

TABLE 4.1: Utility Requirements

Utility	Requirement
Electricity	160,000 kWh
Water	315 L
Sewage	30,000 L

4.3 Methods of Minimizing Energy Requirements and Utilities

To minimize energy requirements and utilities, we decided on a non-traditional disposable approach rather than a traditional stainless steel approach. Previous industrial studies have indicated that energy requirements and utilities are minimized when using the disposable approach over the stainless-steel approach [28]. For example, disposable-based facilities consume 87% less water than stainless steel based facilities. [28]. Furthermore, for stainless steel-based facilities, nearly 900 kg of steam is required per batch to sterilize operations. However, for disposable single-use bags, there is no need for clean steam. Finally, disposable-based facilities result in an electrical saving of approximately 30%, making it the optimal choice.

Using this new insect cell technology also minimizes utilities compared to the egg-based approach, as shown in Figure 4.1. Building utilities decrease by nearly 51%, and process utilities decrease by 92% [15].

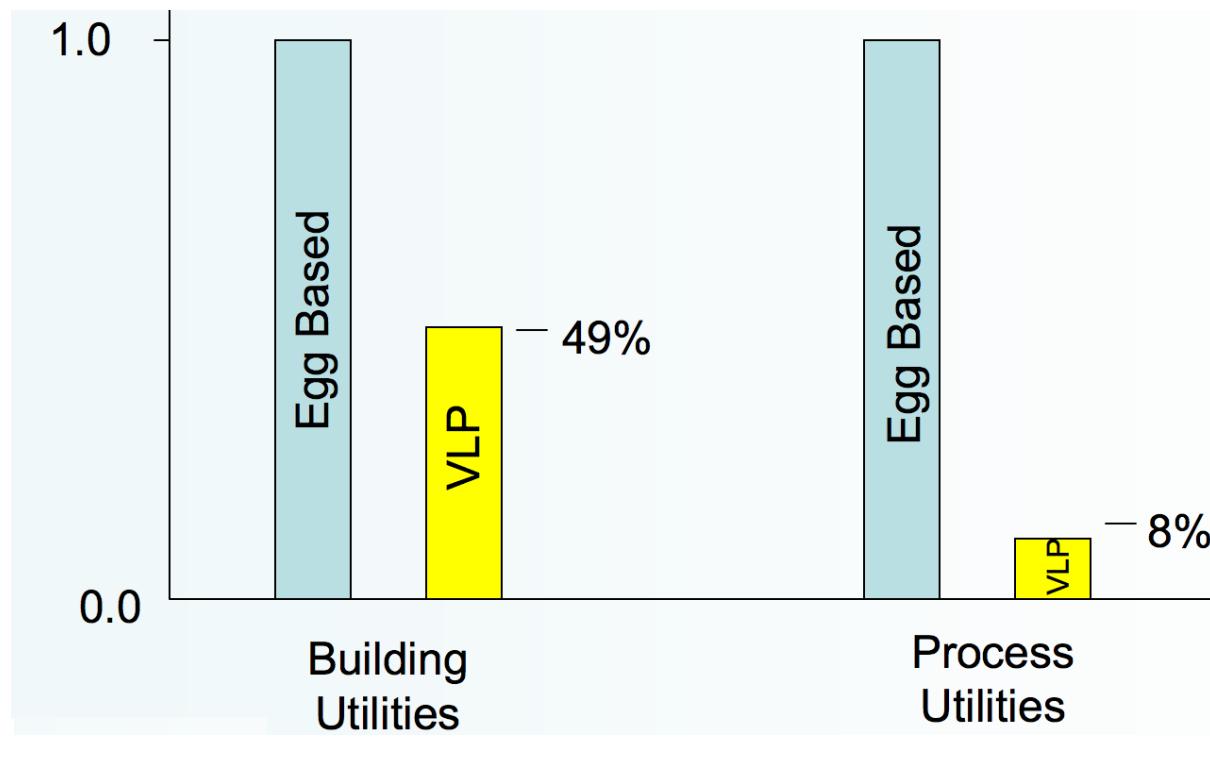


FIGURE 4.1: Using this new insect cell technology also minimizes utilities compared to the egg-based approach. Building utilities decrease by nearly 51%, and process utilities decrease by 92% [15].

Equipment List and Unit Descriptions

As shown in the process flow diagram in Figure 2.2, numerous types of equipment are necessary to successfully produce trivalent influenza vaccine. To decide upon the specific sizes and types of equipment used, various assumptions were made.

5.1 Assumptions Used

The following assumptions were made to decide upon which equipment was necessary:

1. For proper aeration, each seed train bioreactor is filled to 60% of maximum capacity [21].
2. For proper aeration, each production bioreactor is filled to 70% of maximum capacity [21].
3. Units would function at the specifications given by manufacturers.

5.2 Equipment List

Using these assumptions, the equipment selected for the production of trivalent influenza vaccine is shown in Table 5.1. Detailed specifications for each of these units described in Table 5.1 is shown in Section 6.

TABLE 5.1: Equipment Used in This Process

Step	Unit	Company
Media and Buffer Preparation	Mixing Tank	Mepaco
Quality Control and Safety	Spectrophotometer	Beckman DU-640
	Autoclave	Tuttnauer 3870EA
	Distiller	Series Model 7000-12
	pH Tester	Milwaukee MW101
	Viscometer	Thermo Scientific HAAKE
Waste Treatment	Kill Tank	Actini EDS-1000
Seed Train	125 mL Flask	Corning
	500 mL Flask	Corning
	3 L Flask	Corning
	36 L Flask	Corning
	200 L Bioreactor	Xcellerex XDR
	2000 L Bioreactor	Xcellerex XDR
Production	2000 L Bioreactor	Xcellerex XDR
Harvesting and Separating	Ultra-centrifuge	Carr UniFuge Pilot
	Ultra-filtration	Sartopure Maxicap
	Chromatography	GE AKATA Avant 150
	Diafiltration	MiniKros Filter
Storage	Liquid Nitrogen Biological Container	MRC

5.3 Unit Description

5.3.1 Seed Train

The seed train is comprised of a 125 mL Corning Flask, a 500 mL Corning Flask, 3 L Corning Flask, 36 L Corning Flask, two Xcellerex XDR 200 L Bioreactors, and finally a Xcellerex XDR 2000 L Bioreactor. Each reactor is designed to include 40% head space, and to ensure the concentration of insect cells increases from 5×10^5 cells/mL to 4×10^6 cells/mL. The flasks were chosen because they are low in cost, disposable, and suited for rapid bacterial growth with adequate shakers to prevent cell death. The bioreactors are also very well suited to growing insect cells with air sparging equipment included, as well as built in systems to monitor the pH and concentration of relevant compounds. They also operate with disposable liners maintaining the disposable nature of our plant.

5.4 Production Bioreactor

An additional Xcellerex XDR 2000 L Bioreactor is used as the production reactor for the same reasons mentioned above.

5.5 Harvesting and Separating

The Carr Unifuge Pilot was selected for the centrifuge because it was designed for use with insect and mammalian cells. The unit has a built in clarification process, is an entirely closed process, exerts low shear stresses on the cells, includes its own pump and occupies less space than traditional units. This unit also utilizes disposable liners. For the first pass, the unit is to be operated at maximum feed rate of 4 L/min, a pressure of 80-100psi, 20°C, and a force of 3000 x g. For the second pass is will be fed at 2 L/min with the same pressure, temperature and force.

Size three Sartopure Maxicaps were selected for the ultrafiltration unit because they are designed for sterile pharmaceutical applications and have demonstrated very reliable operation. They are also self contained and include a non-traditional geometry which prevents deterioration over time. For the first pass, they are to be operated with a feed rate of 3.8 L/min, a pressure of 40 psi, 20°C and approximately a 10-minute hold up time. For the second pass, they will be fed at 1.9 L/min with the other conditions remaining the same.

The GE Healthcare Life Sciences AKTA avant 150 unit was selected for the chromatography column with Capto Q High Scale 26/20 chromatography columns and Capto Q ion exchange media. This unit includes its own pump and optimized operating system called UNICORN6 which automates buffer preparation and monitors pH. The ion exchange media has been demonstrated to have a very high recovery and is readily scalable for this function. The unit will be run with 2 columns in parallel being fed at 200 mL/min combined, a pressure of 725 psi, 20°C, and an elution buffer of 1.5 M NaCl (pH 7).

For the diafiltration unit, the MiniKros Filter Modules were selected because they are relatively low cost, with a high packing density, consistent performance and tangential flow design to prevent clogging of the pores. They will be fed at 1.5 L/min, a pressure of 30 psi, 20°C, and a hold up time of approximately 2 1/2 minutes.

Equipment Specification Sheets

As shown in the process flow diagram in Figure 2.2, numerous types of equipment are necessary to successfully produce trivalent influenza vaccine. The specification sheets for each piece of equipment are shown below.

6.1 Buffer and Media Preparation

Buffer Mixing Tank		
Identification:	Item	<i>Mepaco Sanitary Tank</i>
	Item No.	A6493
	No. Required	1
Function	Store and mix buffer solution	
Operation	Batch	
Materials:	Water: 15 L per batch	
Handled	ADA Buffer: 15 mol per batch	
Design Data	Pressure: 14.7 psi Temperature: 27°C	
Utilities	Power Requirements: 15 kW	
Media Mixing Tank		
Identification:	Item	<i>Mepaco Sanitary Tank</i>
	Item No.	A6493
	No. Required	1
Function	Store and mix media	
Operation	Batch	
Materials:	Buffer Solution: 15 L per batch	
Handled	SF-900 III SFM Media: 1,300 L per batch	
Design Data	Pressure: 14.7 psi Temperature: 27°C	
Utilities	Power Requirements: 15 kW	

6.2 Seed Train

125 mL Bioreactor		
Identification:	Item	<i>Corning 125 mL Spinner Flask</i>
	Item No.	3152
	No. Required	1
Function	Allows for cell growth	
Operation	Fed-Batch	
Materials Handled	Cell Culture-Media Mixture: 40 mL per batch	
Design Data	Pressure: 14.7 psi Temperature: 27°C pH: 6.2	
Utilities	Power Requirements: 130 W	

500 mL Bioreactor		
Identification:	Item	<i>Corning 500 mL Spinner Flask</i>
	Item No.	3153
	No. Required	1
Function	Allows for cell growth	
Operation	Fed-Batch	
Materials Handled	Cell Culture-Media Mixture: 320 mL per batch	
Design Data	Pressure: 14.7 psi Temperature: 27°C pH: 6.2	
Utilities	Power Requirements: 130 W	

3 L Bioreactor		
Identification:	Item	<i>Corning 3 L Spinner Flask</i>
	Item No.	CLS3563-4EA
	No. Required	1
Function	Allows for cell growth	
Operation	Fed-Batch	
Materials Handled	Cell Culture-Media Mixture: 2.5 L per batch	
Design Data	Pressure: 14.7 psi Temperature: 27°C pH: 6.2	
Utilities	Power Requirements: 130 W	

36 L Bioreactor		
Identification:	Item	<i>Corning 36 L Spinner Flask</i>
	Item No.	CLS450036L-1EA
	No. Required	1
Function	Allows for cell growth	
Operation	Fed-Batch	
Materials Handled	Cell Culture-Media Mixture: 21 L per batch	
Design Data	Pressure: 14.7 psi Temperature: 27°C pH: 6.2	
Utilities	Power Requirements: 130 W	

200 L Bioreactor		
Identification:	Item	<i>Xcellerex XDR 200 L Single-Use Bioreactor</i>
	Item No.	29-0929-27
	No. Required	2
Function	Allows for cell growth	
Operation	Fed-Batch	
Materials Handled	Cell Culture-Media Mixture: 160 L per batch	
Design Data	Pressure: 14.7 psi Temperature: 27°C pH: 6.2	
Utilities	Power Requirements: 230 V, 60 Hz	

2000 L Bioreactor		
Identification:	Item	<i>Xcellerex XDR 2000 L Single-Use Bioreactor</i>
	Item No.	29-0929-28
	No. Required	1
Function	Allows for cell growth	
Operation	Fed-Batch	
Materials Handled	Cell Culture-Media Mixture: 160 L per batch	
Design Data	Pressure: 14.7 psi Temperature: 27°C pH: 6.2	
Utilities	Power Requirements: 230 V, 60 Hz	

6.3 Production

2000 L Production Bioreactor	
Identification:	Item <i>Xcellerex XDR 2000 L Single-Use Production Bioreactor</i> Item No. 29-0929-29 No. Required 1
Function	Allows for virus to attack cells and reproduce
Operation	Fed-Batch
Materials Handled	Cell Culture-Media Mixture: 1300 L per batch
Design Data	Pressure: 14.7 psi Temperature: 27°C pH: 6.2
Utilities	Power Requirements: 230 V, 60 Hz

6.4 Harvesting and Separating

Ultra-Centrifuge	
Identification:	Item <i>CARR UniFuge Pilot</i> Item No. 63303 No. Required 1
Function	Pellet out majority of cells
Operation	Fed-Batch
Materials Handled	Virus-Media Mixture: 4 L/min (First Pass) Virus-Media Mixture: 2 L/min (Second Pass)
Design Data	Pressure: 80-100 psi Temperature: 27°C
Utilities	Power Requirements: 120/230 VAC, 50/60 Hz G-Force: 3000 x g

Ultra-Filtration	
Identification:	Item <i>Sartopure MaxiCaps</i> Item No. 5555305P3 No. Required 2
Function	Remove cells and other impurities from media mixture
Operation	Fed-Batch
Materials Handled	Virus-Media Mixture: 3.8 L/min (First Unit) Virus-Media Mixture: 1.9 L/min (Second Unit)
Design Data	Pressure: 40 psi Temperature: 20°C
Utilities	Power Requirements: 800 W

Chromatography Column		
Identification:	Item	<i>GE Healthcare Life Sciences AKTA avant 150 unit</i>
	Item No.	28-9763-37
	No. Required	1
Function	Separate virus from mixture using an elution buffer	
Operation	Fed-Batch	
Materials Handled	Virus-Media Mixture: 200 mL/min	
Design Data	Pressure: 725 psi Temperature: 20°C pH: 7	
Utilities	Power Requirements: 800 W	
Diafiltration		
Identification:	Item	<i>MiniKros Filter Modules</i>
	Item No.	C02-S010-05-P
	No. Required	1
Function	Filter out sodium chloride and other impurities from virus	
Operation	Fed-Batch	
Materials Handled	Virus-Sodium Chloride Solution Mixture: 1.5 L/min	
Design Data	Pressure: 30 psi Temperature: 20°C	
Utilities	Power Requirements: 800 W	

6.5 Inactivating

Inactivation Batch Reactor		
Identification:	Item	<i>Mepaco/Sanitary Tank</i>
	Item No.	A6493
	No. Required	1
Function	Inactivate virus using formaldehyde	
Operation	Fed-Batch	
Materials Handled	Virus-Sodium Chloride Solution Mixture: 2 L/min	
Design Data	Pressure: 14.7 psi Temperature: 20°C	
Utilities	Power Requirements: 15 kW	

6.6 Storage

Storage Biocontainer		
Identification:	Item	<i>Pall Allegro 2D Biocontainer</i>
	Item No.	LGRTTE200L
	No. Required	1
Function	Store stabilized vaccine	
Operation	Fed-Batch	
Materials Handled	Stabilized Vaccine: 1.5 L/min	
Design Data	Pressure: 14.7 psi Temperature: -80°C	

Biological Container		
Identification:	Item	<i>MRC Liquid Nitrogen Biological Container</i>
	Item No.	CRY-10-50L
	No. Required	1
Function	Store biocontainers containing stabilized vaccine	
Operation	Batch	
Materials Handled	Pall Allegro 2D Biocontainer	
Design Data	Pressure: 14.7 psi Temperature: -80°C	

6.7 Testing and Quality Control

Spectrophotometer		
Identification:	Item	<i>Beckman DU-640 Spectrophotometer</i>
	Item No.	9995.B446.001
	No. Required	1
Function	Monitor sterility and concentration of cells throughout production process	
Operation	Batch	
Materials Handled	5 mL sample of Cell Culture-Media Mixture	
Design Data	Pressure: 14.7 psi Temperature: 20°C	
Utilities	Power Requirements: 120 VAC, 50/60 Hz, 15A	

Autoclave		
Identification:	Item	<i>Tuttnauer Large Capacity Fully Automatic Autoclave</i>
	Item No.	3870EA
	No. Required	1
Function	Sterilize Plant Equipment	
Operation	N/A	
Materials Handled	Plant equipment that will fit in the 15" x 30" autoclave chamber	
Design Data	Pressure: 14.7 psi Temperature: 100°C – 134°C	
Utilities	Power Requirements: 3000 W, 13.6 A	

Distiller		
Identification:	Item	<i>Tuttnauer Large Capacity Distiller Model 7000-12</i>
	Item No.	HEI-023211180
	No. Required	1
Function	Prepare sterile water	
Operation	Continuous	
Materials Handled	Water: 15 L/h	
Design Data	Pressure: 14.7 psi Temperature: 27°C	
Utilities	Power Requirements: 115 V, 60 Hz Water: 15 L/h	

pH Tester		
Identification:	Item	<i>Milwaukee MW101 pH Tester</i>
	Item No.	MW101
	No. Required	1
Function	Monitor pH of mixtures throughout production process	
Operation	Batch	
Materials Handled	5 mL sample of Cell Culture-Media Mixture	
Design Data	Pressure: 14.7 psi Temperature: 27°C	
Utilities	Power Requirements: Battery Included	

Viscometer	
Identification:	Item Thermo Scientific HAAKE High-Range Portable Viscometer Item No. EW-08705-02 No. Required 1
Function	Monitor viscosity of mixtures throughout production process
Operation	Batch
Materials Handled	50 mL sample of Cell Culture-Media Mixture
Design Data	Pressure: 14.7 psi Temperature: 27°C
Utilities	Power Requirements: Four AA Batteries Included

Equipment Cost Summary

7.1 Methods for Estimating Equipment

Each individual piece of equipment in the process was sized and priced according to material and energy balances. Since the equipment used in this facility is primarily disposable, most of its cost is included in variable costs rather than capital costs.

7.2 Equipment Cost

Equipment must be costed for each step in this process:

1. Media and buffer preparation
2. Quality control and safety
3. Waste treatment
4. Seed train and production reactor
5. Harvesting and separating
6. Inactivating
7. Storing

7.2.1 Media and buffer preparation

Two mixing tanks are required for media and buffer preparation. The freight on board (FOB) cost for each mixing tank is \$20,000. Accounting for installation costs, the total equipment cost for media and buffer preparation is \$120,000.

7.2.2 Quality Control and Safety

The equipment costs for quality control and safety are shown in Table 7.1. This includes the costs for the spectrophotometer, the autoclave, the pH tester, the viscometer, and the distiller.

TABLE 7.1: Equipment Cost of Quality Control and Safety

Unit	FOB Cost (\$)	Cost After Installation
Beckman DU-640 Spectrophotometer	5,000	15,000
Tuttnauer 3870EA Large Capacity Fully Automatic Autoclave	15,000	45,000
Tuttnauer Series Large Capacity Distillers Model 7000-12	8,000	24,000
Milwaukee MW101 pH Tester	800	2,400
Thermo Scientific HAAKE High-Range Portable Viscometer	3,000	9,000

7.2.3 Waste Treatment

One Actini EDS-1000 kill tank is required to treat the liquid waste. The FOB cost of this tank is \$200,000, and the cost after installation is \$600,000.

7.2.4 Seed Train and Production Reactor

The equipment costs for the seed train and production reactors are shown in Table 7.2. Since the 125 mL, and 500 mL, 3 L, and 36 L flasks are disposable, these costs are considered variable costs.

TABLE 7.2: Equipment Cost of Seed Train and Production

Unit	FOB Cost (\$)	Cost After Installation
Xcellerex XDR 200 L Single-Use Bioreactor	40,000	120,000
Xcellerex XDR 2000 L Single-Use Bioreactor	300,000	900,000
Xcellerex XDR 2000 L Single-Use Production Bioreactor	300,000	900,000

7.2.5 Harvesting and Separating

The equipment costs for harvesting and separating are shown in Table 7.3.

TABLE 7.3: Equipment Cost of Harvesting and Separating

Unit	FOB Cost (\$)	Cost After Installation
Carr UniFuge Pilot	60,000	180,000
Sartopure Maxicap	2,800	8,400
Chromatography	100,000	300,000

7.2.6 Inactivation

One mixing tank is required for inactivation. The FOB cost for the mixing tank is \$20,000. Accounting for installation costs, the total equipment cost for inactivation is \$60,000.

7.2.7 Storage

Two cryogenic containers are needed to store the Pall biocontainers. The FOB cost for each of these containers is \$20,000. Accounting for installation costs, the total equipment cost for storage is \$120,000. A summary of the equipment costs are shown in Table 7.4. In total, \$3.3 million is required for equipment costs. A breakdown of the equipment costs is shown in Figure 7.1. A detailed analysis of these costs is shown in Appendix B.

TABLE 7.4: Equipment Cost Summary

Process	Equipment Cost (\$)
Media	60,000
Quality Control and Safety	95,400
Waste Treatment	600,000
Seed Train and Production Reactor	1,920,000
Harvesting and Separating	490,000
Inactivation	60,000
Storage	120,000

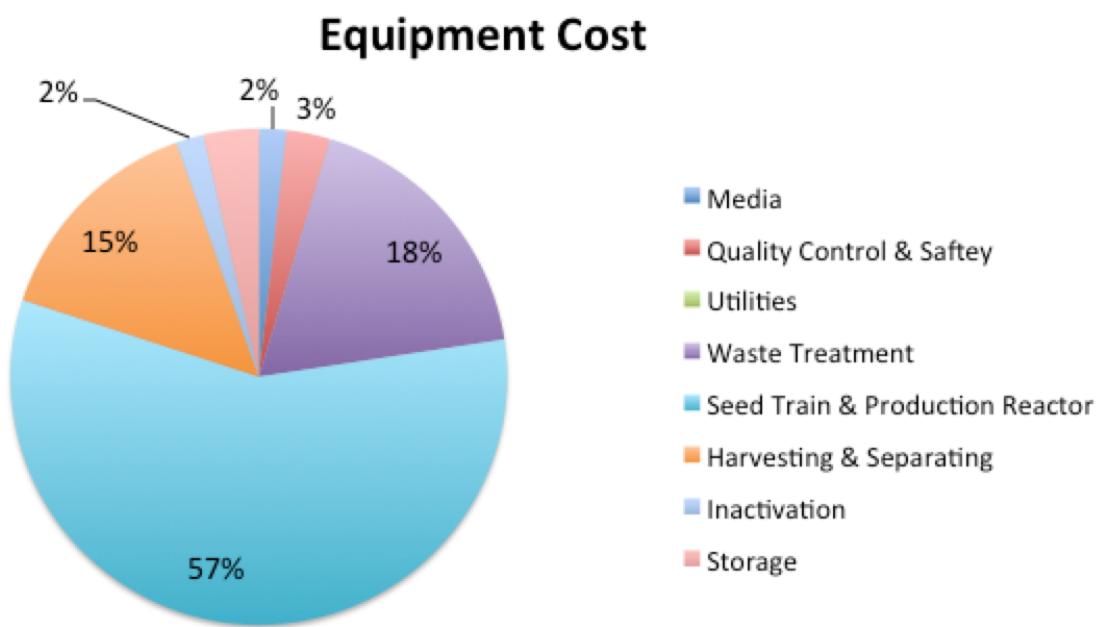


FIGURE 7.1: A breakdown of the equipment costs.

Fixed-Capital Investment Summary

The fixed capital costs of the plant include the cost of the land under the plant, the cost of building the plant, the cost of equipment to run the plant and the working capital for the plant. The land under the plant is expected to cost about \$1,000,000 for two acres of land [29]. This land will provide enough space for the 90,000 ft² building, a parking lot, as well as space for any additional facilities deemed necessary (i.e. picnic benches for employees to take their breaks). The building is expected to cost about \$18,000,000, which is based on the cost to build a 1 story warehouse in Chicago, IL using “open shop” labor with bearing walls and no basement multiplied by a factor of 1.75 to account for the second story. The cost per square foot is about \$115, 25% of which is contractor fees and 7% in architectural fees with the remaining 68% including the labor and material costs [29]. A working capital of \$2,000,000 was selected to account for the cyclical nature of the vaccine market. This is not only to ensure in the event a piece of equipment breaks the funding to replace it is available, but also to account for the purchasing of all the disposables and raw materials at the beginning of production season without seeing any of the profit from it until production is completed or near completion.

Safety, Health, and Environmental Considerations

9.1 Safety and Health Considerations

Vaccine production requires numerous safety and health considerations. The safety and health considerations include: 1) maintaining a sterile environment throughout our process; 2) controlling and isolating activated virus; 3) properly storing inactivated virus; 4) avoiding risk of contamination and cross-contamination throughout the process; 5) maintaining a safe environment for all employees; and 6) meeting good manufacturing practices.

To ensure sterility and quality, influenza vaccines made in this new manufacturing facility will be produced using Good Manufacturing Practices (GMP) as laid out by the FDA and undergo batch testing for purity and potency. Since we are looking for FDA licensure, the produced vaccine must meet rigorous standards of efficacy and safety, and its potential benefits in preventing influenza must clearly outweigh any risks [30]. In assuring the production of a sterile vaccine, safety and quality-control practices will also be maintained. Most importantly, activated virus used throughout the production process will be in a controlled, isolated area to ensure containment of the virus. In order to avoid risk of contamination or adulteration in the process including carryover between unit operations or batches, cross-contamination between products, and the introduction of contaminants from the environment, raw materials, or from inadequate cleaning, closure analyses will be performed. A closure analysis will entail a systematic evaluation of the risk in each process step based on the process control level required and the closure level used for particular connections. Leveraging closed systems and maximizing the use of disposable systems mitigates contamination risks and allows for a safer environment [31].

In addition, safety of all employees must be taken into careful consideration. All employees on site will take part in mandatory safety training as laid out by the Occupational Safety and Health Administration (OSHA) Standards and Training Guidelines. This will entail hands-on training in the recognition, avoidance, and prevention of safety hazards on the job site [32]. Specifically, procedures for safe decontamination of waste and equipment as well as emergency procedures for events such as spillages will be well documented and reviewed periodically.

9.2 Environmental Considerations

It is also essential that any environmental impacts caused by this manufacturing facility be analyzed and mitigated. The solid, liquid, and gas wastes generated by this facility will be properly disposed of. Gas emissions from the facility are relatively minimal and consist primarily of carbon dioxide at levels acceptable for release into the atmosphere. The solid waste generated consists primarily of disposable equipment linings and single-use separation units. It will be treated by off site incineration by Waste Management, Inc. The liquid waste generated from the facility consists primarily of cell and media waste, formaldehyde, and sodium hydroxide. This waste is the most significant to consider and will be treated with a kill tank. These proper waste management techniques will reduce the impact of the facility on the environment.

The environmental impact of our trivalent flu vaccine production plant plays a large role in the design and bottom line of the process. Therefore, to evaluate the environmental contributions of the process, we completed an environmental life-cycle assessment on both the traditional stainless-steel approach, and the non-traditional disposable approach. To go forward with the disposable approach, we made sure this method has a smaller environmental impact.

We considered four energy consumption sources — materials, sterilization, cleaning, and waste disposal — in each step of our process (for a detailed description of our process, see Chapters 2 and 3). To do this analysis, we obtained energy conversion data on the various energy consumption sources, as shown in Tables 9.1, 9.2, and 9.3. This data was collected from GE healthcare, and Rawlings *et al* [33, 34].

TABLE 9.1: Materials Used in the Process

Component	Life span (number of cycles)	Component Weight (kg)	Material
Bioreactor tanks	600	420	Stainless steel
Bioreactor linings	1	2	Polypropylene
Flexible tubing	1	2	Polypropylene
Spectrophotometer	600	190	Stainless steel
Autoclave	600	190	Stainless steel
Distiller	600	150	Stainless steel
Viscometer	600	150	Stainless steel
Carbon filter	1	0.5	Polypropylene
Biocontainer	1	2	Polypropylene
Ultracentrifuge	600	420	Stainless steel
Ultracentrifuge liner	1	2	Polypropylene
Ultrafiltration caps	1	20	Polypropylene
Chromatography column	600	420	Stainless steel
Chromatography cap	1	23.6	Polypropylene
Diafiltration filter	1	2	Polypropylene
Waste treatment kill tank	600	420	Stainless steel
Mixing tank	600	420	Stainless steel

TABLE 9.2: Energy Conversion Data for the Material Energy Consumption Source

	Stainless Steel	Polypropylene
Raw Material Energy Value (MJ/kg)	70	81
Material Treatment (MJ/kg)	30	-
Welding (MJ/kg)	0.6	-
Extrusion molding (MJ/kg)	-	7

TABLE 9.3: Energy Conversion Data for the Sterilization and Waste Management Energy Consumption Sources

	Sterilization	Waste Management
Gamma Radiation (kGy)	25	-
Liquid Waste Management (kWh)	-	7

Furthermore, we made the following justifiable assumptions:

1. Plastic components were made of polypropylene only (even if they possibly contained other polymers).
2. Liquid filters are replaced after every batch.
3. Stainless steel mixing tanks have a lifetime of 600 batches.
4. Gamma irradiation is used to sterilize the disposable system.
5. Negligible energy is necessary for cleaning (since a disposable system is used).

Using the relevant data and assumptions, we calculated that the energy consumption for materials, sterilization, and waste disposal is 5,100 MJ/batch, 32 MJ/batch, and 1,800 MJ/batch, respectively. For twenty-one batches per year and a plant life of eight years, the full life-cycle energy consumption for materials, sterilization, and waste disposal is 730 GJ, 5 GJ, and 261 GJ, respectively (Figure 9.1).

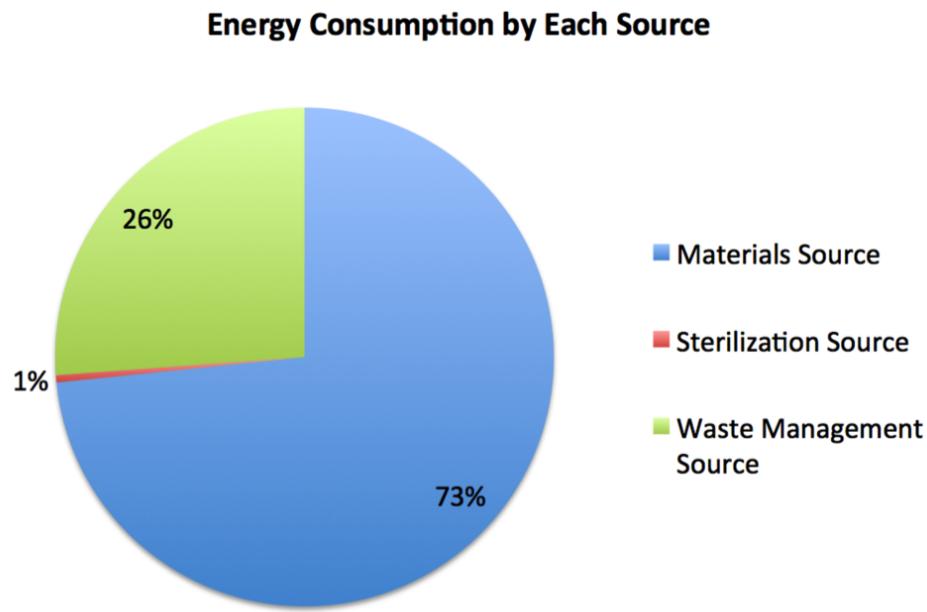


FIGURE 9.1: The energy consumption for materials, sterilization, and waste disposal is 5,100 MJ/batch, 32 MJ/batch, and 1,800 MJ/batch, respectively..

As expected, the energy consumption per batch for the disposable approach (7,000 MJ/-batch) is less than the energy consumption per batch for the stainless steel approach (8,000 MJ/batch). Overall, the disposable approach has a smaller environmental impact and a greater financial benefit than a conventional stainless steel system, making it the preferred method in our process.

Other Important Considerations

Several other design features of the plant were considered, including: 1) the controllability of the process; 2) the timeline for getting the plant to full production; 3) the strategic layout of the plant; 4) the most efficient scheduling of the equipment; and 5) the labor necessary, and the corresponding scheduling.

10.1 Process Controllability and Instrumentation

The Beckman DU-640 Spectrophotometer, Tuttnauer 3870EA Large Capacity Fully Automatic Autoclave, Tuttnauer Large Capacity Distiller model 7000-12, Milwaukee MW101 pH Tester, and Thermo Scientific HAAKE Viscometer were all selected to assist in the quality control and safety testing performed throughout the plant. The spectrophotometer is necessary for the continuous monitoring of the concentration of cells in each of the batch reactors. If the concentration becomes too high the cells will begin to die due to a lack of available nutrients, so this must be closely monitored by checking the optical density of a sample of media in the spectrophotometer. This specific model was selected because it is a stand-alone unit with a wide range of applications and is designed for ease of use. The autoclave is necessary for cleaning every single piece of equipment that comes in contact with the process without a disposable liner. The unit is used to sterilize equipment, and this model in particular is large enough to accommodate the plant's needs, is fully automated and includes an integrated printer for cycle documentation. The distiller is necessary for any buffer preparations, dilutions and other miscellaneous uses in the lab and plant in order to cut down on the cost of purchasing water for injection, which is used in preparing the media. This model was selected because of the very large volume it can prepare and its direct connection to the water supply for continuous use. Finally the viscometer and pH meter are necessary to test samples of the media because the viscosity of the mixture effects the shear stresses applied to the cells throughout the process and too high or low of a pH will denature the virus-like protein irreversibly. The bioreactors

monitor these levels however measurements also need to be taken of the solutions in the flasks and after samples have been removed from the reactors in some cases. These models were selected because in both cases they are portable and highly accurate over a large range.

10.2 Startup Considerations

Another important factor in moving forward with this design is the timeline from our current design to full production. Therefore, the following steps will be necessary before reaching full capacity:

1. Construction of our plant.
2. Commissioning and qualification of our products.
3. Validation of our process.

Biotechnology industry has demonstrated that compared to egg-based processes, insect cell culturing takes a shorter time to startup [15]. Insect cell-culture processes save approximately two years during startup, another reason why we went forward with this novel technique (Figure 10.1).

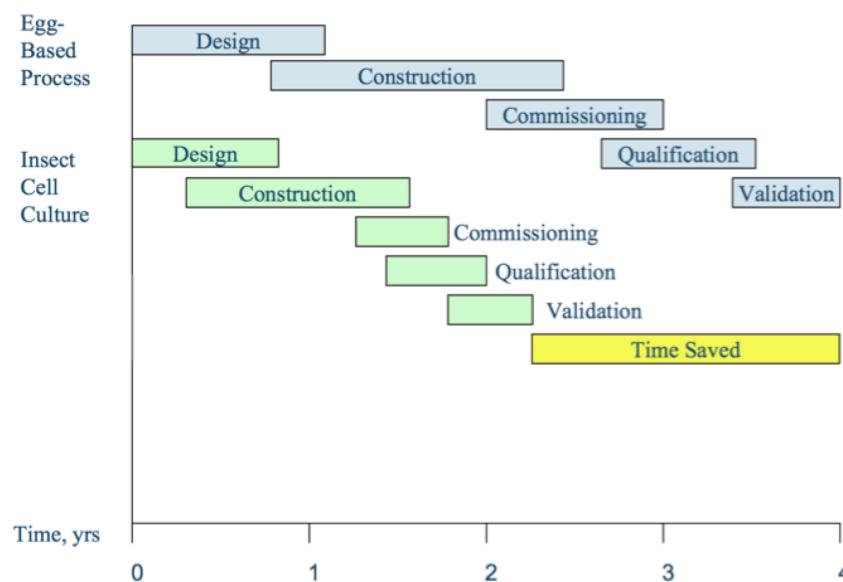


FIGURE 10.1: Startup considerations were made when choosing between the disposable approach and the stainless-steel approach. Nearly two years in startup are saved using the disposable approach [15].

Construction is estimated to take approximately one year, followed by a year-long commissioning and qualification. By the commissioning phase, all of the employees will need in order to run the plant and produce about a quarter of the full capacity of the plant. The product made during this time will be used for testing by various government agencies for approval, so there will not be any income in this year.

The next step will be to carry out a pilot study to determine the optimal operating conditions for each piece of equipment during the validation phase. The conditions presented in Section 6 are based on specifications provided by the manufacturers, so these values should be refined. After employing some or all of the engineers for the plant, they will be sent to each manufacturer to test these operating conditions, as well as to prepare protocols for the operators who will be running each of the machines. By the end of this validation phase, full production of 50 million doses per year will be possible.

However, since the new vaccine is first being introduced into the market during the first year of production, the plant will run at half capacity. The following year 3/4 of full capacity is expected and in the third year of production the plant should reach full capacity although these values will vary with market demand and should be re-evaluated prior to each production season.

10.3 Plant Layout

In designing the layout of the plant, safety of the personal and product were the main priorities, as well as minimizing the size of the building to lower costs. A two story building that is 200 feet by 450 feet was select, which requires a property of at least 90,000 feet². Since parking for employees and a security entrance are required, a property of 100,000 feet² is needed.

The top floor will be used for offices, while the first floor includes the plant and lab. The layout of the plant and lab are shown in Figure 10.2.

One of the primary safety features of this layout is the three-corridors, which will assist in isolating the virus from unnecessary contact with personnel. These corridors include the:

1. Employee/Personnel Corridor
2. Main Corridor

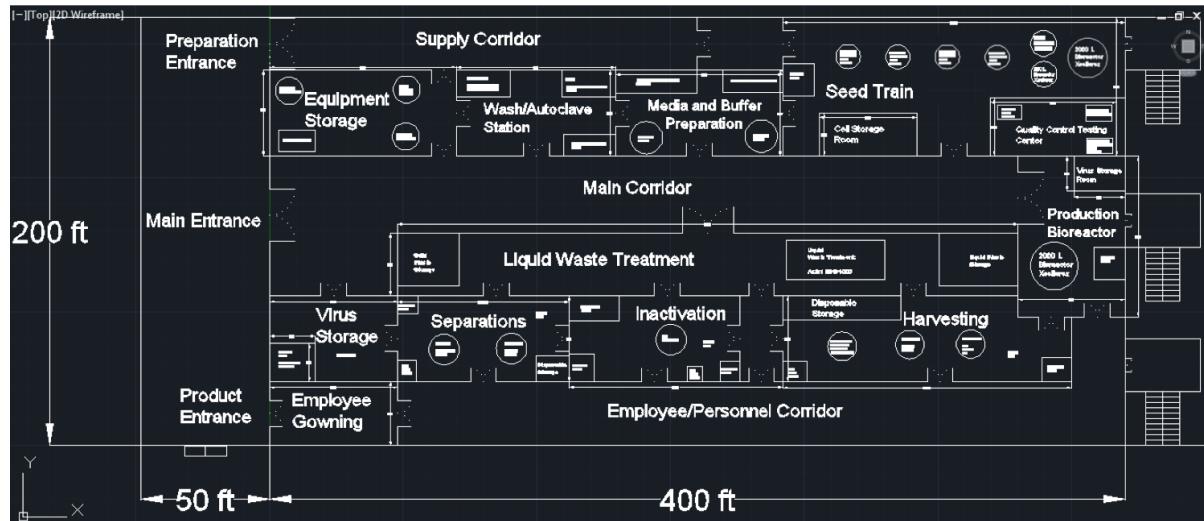


FIGURE 10.2: The first floor includes the plant and lab.

3. Supply Corridor.

The Employee/Personnel Corridor has a gowning station at its entrance, so any employees entering rooms with active virus will enter through here. The main corridor will be used for delivery of equipment and removal of waste. Finally the supply corridor will be used for delivery of raw materials.

Each section of the plant described in the process flow diagram in Figure 2.2 has its own room in the plant. This is to ensure each process that includes active virus is isolated from those that do not as well as to maintain each room at the appropriate temperature for the processes taking place in the room. In addition there is a Wash/Autoclave Station where all of the equipment used in the plant will be sterilized after use. The seed train room also includes smaller rooms for cell storage and the quality control testing center. The cell storage room is essentially a large freezer, which will be maintained at -20° C to stabilize the cells until they are ready to be used. The quality control testing center is where samples of the media in each bioreactor will be taken to determine the concentration of cells suspended in the media as well as testing of the final product to ensure the quality is maintained. The room will include a spectrometer, pH meter and viscometer as well as the necessary glassware and other lab equipment. Each room that has equipment using disposable liners has space designated for storing the liners prior to use as well as solid and liquid disposal. At the end of each day, the liquid batch is moved to the liquid waste treatment room, while the solid waste is picked up by Waste Management, Inc.

10.4 Process Scheduling

In order to ensure the most efficient operation of the plant a schedule for the equipment operation was put together. In Figure 10.3, a schedule of the first 30 days of operation are shown.

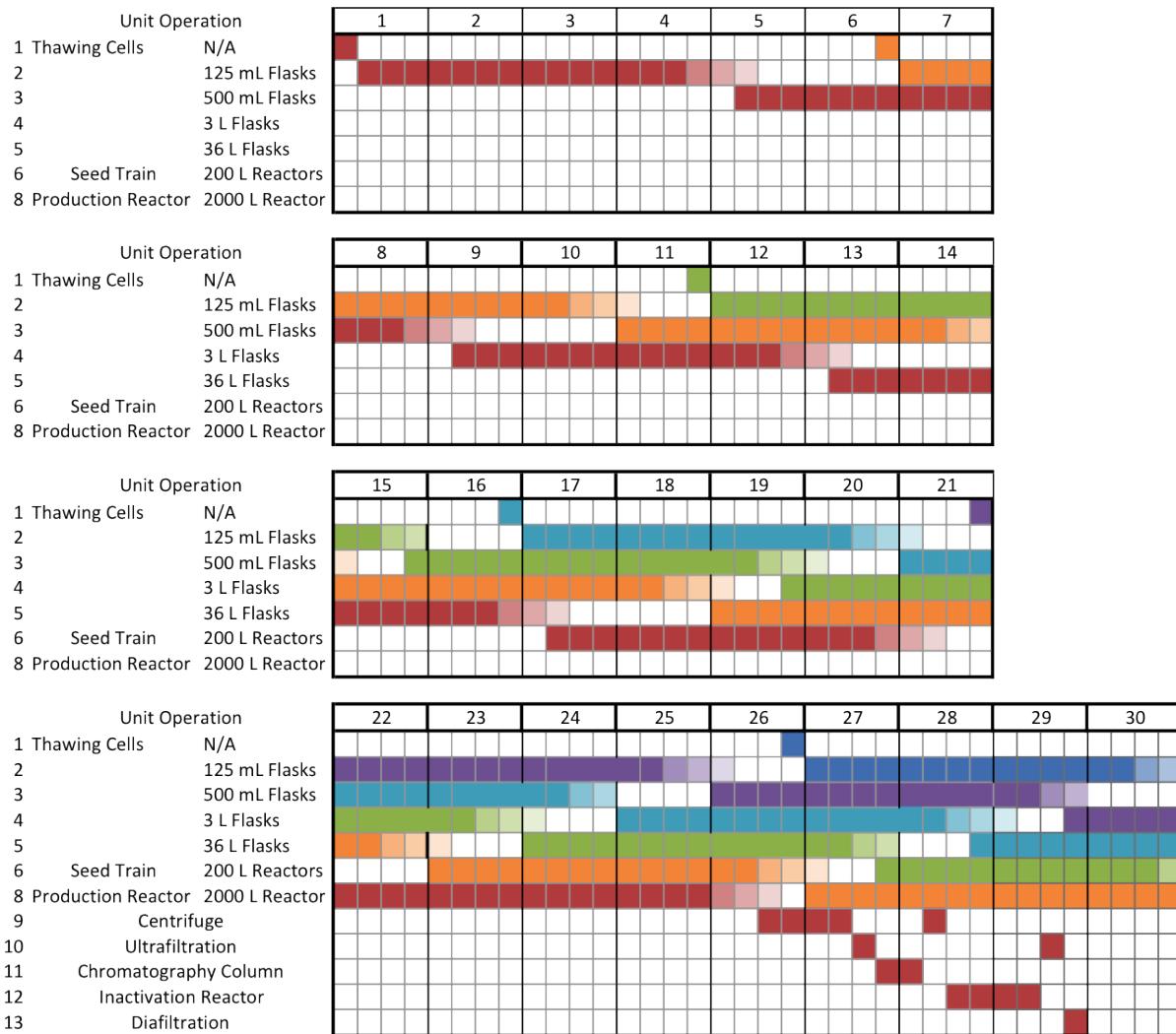


FIGURE 10.3: In order to ensure the most efficient operation of the plant a schedule for the equipment operation was put together.

Each block represents a 6-hour period and each color represents a different batch. A new batch is started every 3 days with the exception of the second batch starting a day later to allow time for trouble shooting any issues that may arise. The time spent in each bioreactor shows the time spent fading out because of the variability in cell growth. It should also be noted that each batch is expected to take 29 days. However, since the

batches will be staggered, each new batch will start approximately every 3 days. As a result, a total of two to three months are required for this process.

10.5 Labor Scheduling

In addition to scheduling the equipment, the personnel needs and shift schedules were designed. The plant will employ 3 laboratory technicians and a laboratory manager who will work a standard workweek of 8 am to 4 pm Monday through Friday. The lab manager will also always be on call incase of any impending needs during nights and weekends. Similarly, the engineering manager and maintenance manager will work standard workweeks and will always be on call. The plant will also employ two administrators, two human resource employees, and four secretaries with standard work weeks.

The engineering manager will oversee 3 engineers who will work 8am to 4pm Monday though Friday with a rotating on call schedule. Each will have one out of every three weekends when they are on call (i.e. from 4pm on Friday to 8am on Monday) and the other weeks they will either be on call Monday and Tuesday night or Wednesday and Thursday night.

In addition to the engineers, the engineering manager will also be in charge of the operators. There will be 8 rover operators employed with two on duty at all times. They work in 8-hour shifts, 12 am to 8 am, 8 am to 4 pm, and 4pm to 12 am 5 days a week. They maintain a weekly schedule with the more senior rover operators working the same shift 5 days in a row with 2 days off. The other 4 rover operators will work the same shift for 2 or 3 days and then work a later shift the following 3 or 2 days with the 2 remaining days off (e.g. Team D works 8 am to 4 pm Monday through Wednesday and 4 pm to 12 am Thursday and Friday with weekends off). In addition, the 8 am to 4 pm shift on Sundays will be open for overtime on a voluntary basis. The 12 control operators employed at the plant work in 12 hour shifts with a 3 week rotating schedule. Each team of two works 3 days in a row and then they have a week off, then they work 4 days in a row with another week off. The difficulty in the scheduling of each of these positions was taken into account in setting the salaries for the operators.

The operating schedules for the managers and office workers are shown in Figure 10.4. The operating schedule for the engineers is shown in Figure 10.5. The operating schedule for the control operators is shown in Figure 10.6. Finally, the operating schedule for the rovers is shown in Figure 10.7.

		Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
	Lab Technicians (3)	Red	Red	Red	Red	Red		
	Management	Orange	Orange	Orange	Orange	Orange		
	Engineering Manager (1)	Orange	Orange	Orange	Orange	Orange		
	Lab Manager (1)	Yellow	Yellow	Yellow	Yellow	Yellow		
	Maintenance Manager (1)							
	Office							
	Administrators (2)	Green	Green	Green	Green	Green		
	Human Resources (2)	Green	Green	Green	Green	Green		
	Secretaries (4)	Green	Green	Green	Green	Green		
		Green	Green	Green	Green	Green		

FIGURE 10.4: The operating schedules for the managers and office workers.

Week 1								
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	
Engineer A	Blue	Blue	Blue	Blue	Blue			
Engineer B	Light Blue							
Engineer C								
Week 2								
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	
Engineer A	Blue	Blue	Blue	Blue	Blue			
Engineer B	Light Blue		Light Blue	Light Blue	Light Blue			
Engineer C								
Week 3								
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	
Engineer A	Blue	Blue	Blue	Blue	Blue			
Engineer B	Light Blue		Light Blue	Light Blue	Light Blue			
Engineer C								

FIGURE 10.5: The operating schedule for the engineers.

Week 1								
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	
Team A (2)	Dark Purple	Dark Purple	Dark Purple					
Team B (2)		Dark Purple	Dark Purple					
Team C (2)			Dark Purple					
Team D (2)				Dark Purple				
Team E (2)					Dark Purple			
Team F (2)	Red							
Week 2								
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	
Team A (2)				Dark Purple	Dark Purple	Dark Purple	Dark Purple	
Team B (2)					Dark Purple	Dark Purple	Dark Purple	
Team C (2)						Dark Purple	Dark Purple	
Team D (2)							Dark Purple	
Team E (2)	Red							
Team F (2)								
Week 3								
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	
Team A (2)	Dark Purple							
Team B (2)		Dark Purple						
Team C (2)			Dark Purple					
Team D (2)				Dark Purple				
Team E (2)					Dark Purple			
Team F (2)	Red							

FIGURE 10.6: The operating schedule for the control operators.

Rover	Operators	Week 1						
		Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
	Team A (2)							
	Team B (2)							
	Team C (2)							
	Team D (2)							

FIGURE 10.7: The operating schedule for the rovers.

Manufacturing Costs

Non-capital manufacturing costs for this plant include the annual costs of:

1. Media and buffers
2. Utilities
3. Quality control and safety
4. Waste treatment
5. Seed train and production reactor
6. Harvesting and separating
7. Inactivating
8. Storing

The operating costs for media and buffers are shown in Table 11.1.

TABLE 11.1: Cost of Media and Buffers

Material	Cost per Batch (\$/batch)	Cost per year (\$/year)
Media	66,300	1,400,000
Buffer	100	2,100

The operating costs for utilities are shown in Table 11.2. This includes the costs for electricity, oxygen, water, and sewage.

TABLE 11.2: Cost of Utilities

Utility	Cost per Batch (\$/batch)	Cost per year (\$/year)
Electricity	400	10,000
Oxygen	175	4,000
Water	15	315
Sewage	5	105

The operating costs for quality control and safety are shown in Table 11.3. this includes the costs for the spectrophotometer, the autoclave, and the distiller.

TABLE 11.3: Cost of Quality Control and Safety

Material	Cost per Batch (\$/batch)	Cost per year (\$/year)
Beckman Cuvettes	70	1,400
Tuttnauer Autoclave	60	1,200
Series Distiller	35	735

The operating costs for waste treatment are shown in Table 11.4. Since liquid waste is treated in the kill tank which runs on electricity, this cost was accounted for in the utilities variable cost.

TABLE 11.4: Cost of Waste Management

Waste	Cost per Batch (\$/batch)	Cost per year (\$/year)
Solid	350	10,500

The operating costs for the seed train and production reactors are shown in Table 11.5. These costs account for the costs of the linings for each flask and bioreactor used.

TABLE 11.5: Cost of Seed Train and Production

Reactor	Cost per Batch (\$/batch)	Cost per year (\$/year)
Corning 125 mL	100	2,400
Corning 500 mL	130	2,800
Corning 3 L	300	6,000
Corning 36 L	2,000	40,000
Xcellerex 200 L	6,000	130,000
Xcellerex 2000 L	10,000	210,000

The operating costs for harvesting and separating are shown in Table 11.6. This includes the costs for each module and cap used in each separation unit.

TABLE 11.6: Cost of Harvesting and Separating

Unit	Cost per Batch (\$/batch)	Cost per year (\$/year)
Carr UniFuge Pilot	7,000	150,000
Sartopure Maxicap	3,000	70,000
Chromatography	30,000	700,000
MiniKros Filter Modules	12,000	200,000

The operating costs for inactivating include formaldehyde use, which is \$18 per batch, or \$540 per year. Finally, the operating costs for storage are shown in Table 11.7.

TABLE 11.7: Cost of Storage

Unit	Cost per Batch (\$/batch)	Cost per year (\$/year)
Liquid Nitrogen	100	2,000
Pall Biocontainer	1,000	20,000

A summary of the annualized variable costs are shown in Table 11.8.

TABLE 11.8: Annualized Cost Summary

Process	Cost per year (\$/year)
Raw Materials	1,500,000
Quality Control and Safety	3,000
Utilities	12,000
Waste Treatment	10,500
Seed Train and Production Reactor	400,000
Harvesting and Separating	1,100,000
Inactivation	540
Storage	32,100

In total, \$3 million is required per year for operating (excluding labor). Since labor costs \$2.6 million per year, the total variable cost is \$5.6 million per year. A breakdown of the annualized variable costs is shown in Figure 11.1. A detailed analysis of these costs is shown in Appendix B.

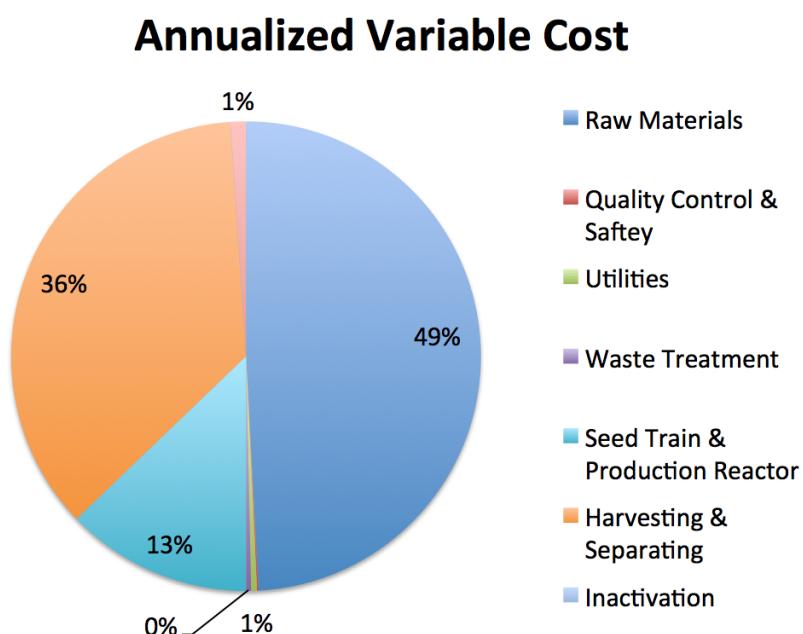


FIGURE 11.1: A breakdown of the annualized variable costs.

Economic Analysis

An economic analysis was performed to evaluate the profitability of this process. Certain assumptions were necessary in order to perform this economic analysis.

12.1 Assumptions Made in Economic Analysis

The following assumptions were made to perform the economic analysis:

1. The building has a straight line depreciation of 5% per year.
2. The plant has a reducing balance depreciation of 15% per year.
3. An aggregate effective tax rate of 22.65% was assumed [35].
4. Shutdown will take one year.
5. Startup takes two years.
6. In the first year of production, the plant will run at 1/2 capacity.
7. In the second year of production, the plant will run at 3/4 capacity.

12.2 Summary of Profitability Measures

Based on these assumptions, it was possible to determine the profitability of the process. Since it takes one year to startup, one year to shut down, and the plant life is eight years, a total of eleven years were analyzed. The cost of good sold is \$0.08 per dose, which is comparable to and less than other biochemical companies producing trivalent vaccine via insect cells [36]. The cumulative cash flow and discounted cash flow is shown in Table 12.1. The resulting cash flow analysis is shown in Figure 12.1.

TABLE 12.1: Economic Analysis: Cash Flow

End of Year	Cumulative Cash Flow (\$1,000)	Discounted Cash Flow (\$1,000)
1	-24,000	-19,000
2	-28,000	-2,300
3	-23,000	2,300
4	-12,000	4,400
5	1,000	4,300
6	13,000	3,200
7	26,000	2,500
8	38,000	2,000
9	50,000	1,600
10	64,000	1,500
11	61,000	-250

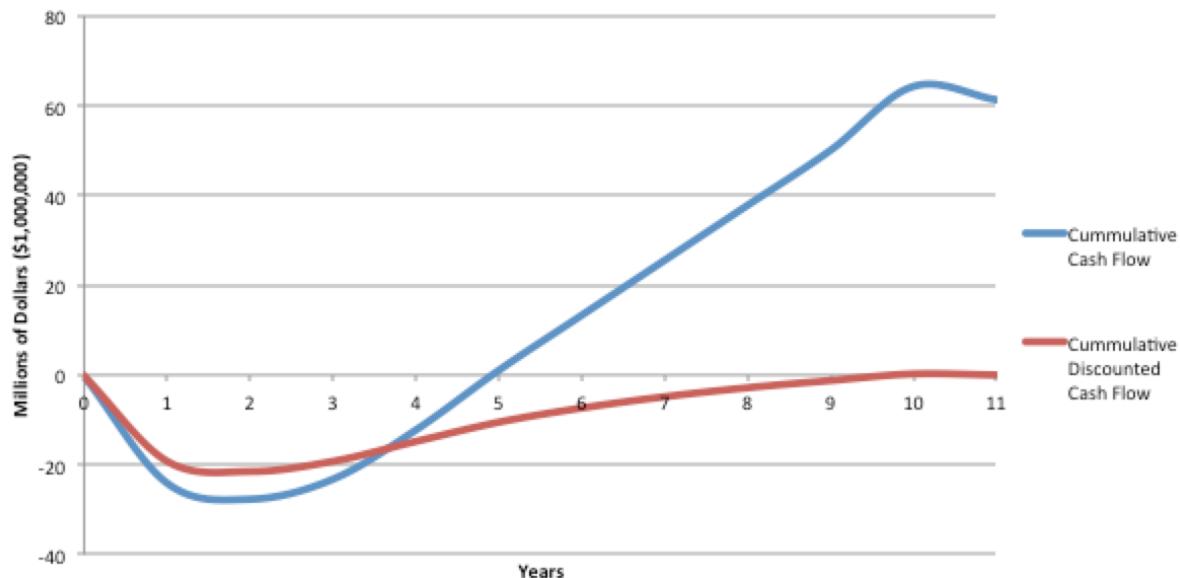


FIGURE 12.1: Cash Flow Analysis.

A dosage price as low as \$0.44/dose is required to obtain a rewarding investment rate of return of 25% and total profit of \$60 million. It will take 5 years to break even. Current egg-based versions of the flu vaccine require a dosage price as high as \$0.64/dose to obtain a similar investment rate of return and total profit [10]. Therefore, this economic analysis was repeated for a dose price of \$0.64 to find the maximum investment rate of return and total profit. The cumulative cash flow and discounted cash flow is shown in Table 12.2 for a dose price of \$0.64. The resulting cash flow analysis is shown in Figure 12.2.

TABLE 12.2: Economic Analysis: Cash Flow

End of Year	Cumulative Cash Flow (\$1,000)	Discounted Cash Flow (\$1,000)
1	-24,000	-17,000
2	-28,000	-1,800
3	-18,000	3,400
4	240	4,600
5	21,800	3,800
6	41,900	2,500
7	61,900	1,800
8	81,800	1,300
9	101,800	900
10	123,800	700
11	118,600	-120

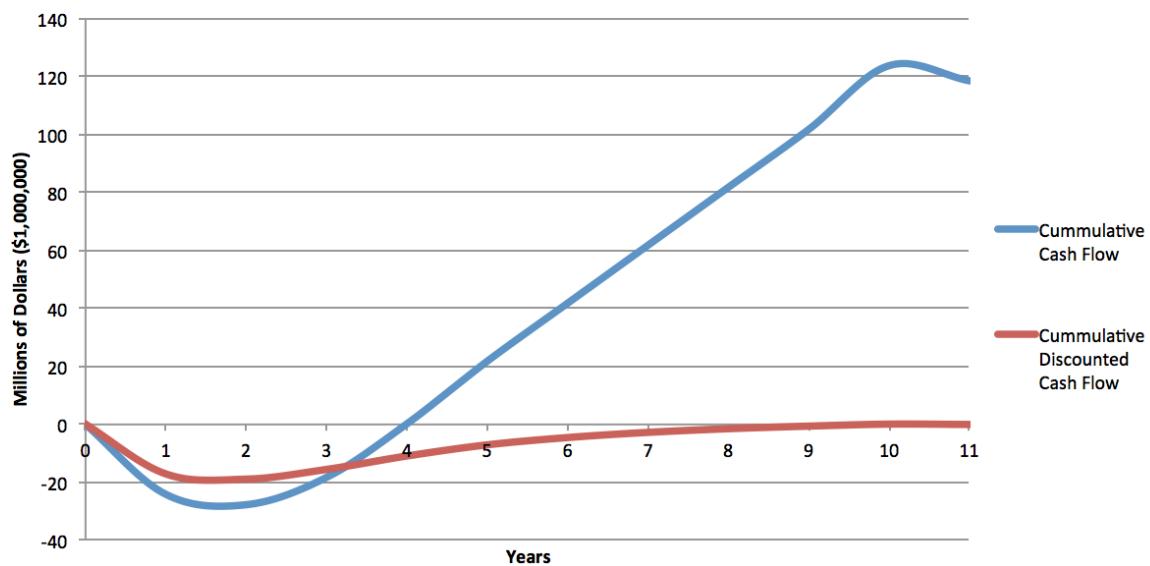


FIGURE 12.2: Cash Flow Analysis.

As shown, a dosage price of \$0.64/dose results in an investment rate of return of 42% and total profit of \$120 million. In this case, it will take four years to break even.

12.3 Investment Rate of Return Sensitivities

Various sensitivities exist for the investment rate of return, including: 1) CDC and FDA regulation changes; 2) market changes; and 3) supply changes.

Conclusions and Recommendations

We seek to produce 50 million doses of flu vaccine, which is approximately one-third of the total flu vaccine distributed during the 2013-2014 influenza season. To develop this trivalent flu vaccine, a non-traditional disposable approach will be taken, with the following steps: 1) preparing media and buffer; 2) expanding cells by a seed train where cells are passaged into larger volumes; 3) producing vaccine by entering the cells into production bioreactors; 4) recovering the contents and products via centrifugation and filtration; 5) inactivating the virus; 6) capturing and purifying the vaccine of interest; and 7) concentrating and stabilizing the material for shipping. For a plant life of 8 years, a dosage price as low as \$0.44/dose is required to obtain a rewarding investment rate of return of 25% and total profit of \$60 million. Current egg-based versions of the flu vaccine require a dosage price as high as \$0.64/dose to obtain a similar investment rate of return and total profit. Therefore, there is the possibility to achieve an investment rate of return as large as 42%, and a total profit of \$120 million. Thus, we recommend going forward with this plant design, and conducting a pilot study.

Acknowledgments

We would like to thank all the professors, mentors, and colleagues who have assisted us in the completion of this project. Specifically, we would like to thank Professors Davis and Okorafor for their guidance, advice, and insight on the steps necessary to successfully design a bioprocess plant. Furthermore, we would like to thank Kristof Toth and Ferdy Budhidharma for their engaging conversation regarding the design of a vaccine production plant.

Appendix A

Material Balance Supplementary Information

See the following page for the Excel sheets on the material balance performed in this study.

	Total doses	5000000									
	3 strains	135 ug/virus/dose									
	1 strain	45 ug virus/dose									
Virus Yield	0.000077 ug virus/cell										
Batches per strain	7										
Production Reactor Recovery	0.80934084										
Seed Reactor Recovery	0.9702										
Inlet Conc	500000 cells/mL										
Outlet Conc	4000000 cells/mL										
Reactor Production	Brand	Media (L)	Min Volume Reactor (L)	Actual Volume (L)	Cost	Entering Conc. (Cells/mL)	Exiting Conc.	Days	Total Cells Entering	Total Cells Exiting	Media Added From Media Tank
Seed Train #6	Xcellerex XDR Single-Use Bioreactor	1329.049	1898.642	2000.000	\$ 300,000.00	4.00E+06	N/A	4.5	5.32E+12	0.000	0.000
Seed Train #5	Xcellerex XDR Single-Use Bioreactor	1329.049	2215.082	2000.000	\$ 300,000.00	5.00E+05	4.00E+06	4	6.65E+11	5.32E+12	1139.191
Seed Train #4	Corning Spinner Flasks	166.131	276.885	200.000	\$ 30,000.00	5.00E+05	4.00E+06	4	8.31E+10	6.65E+11	142.404
Seed Train #3	Corning Spinner Flasks	20.766	34.611	200.000	\$ 30,000.00	36.000	\$ 2,019.90	4	1.04E+10	8.31E+10	17.806
Seed Train #2	Corning Spinner Flasks	2.596	4.326	3.000	\$ 272.50	5.00E+05	4.00E+06	4	1.30E+09	1.04E+10	2.231
Seed Train #1	Corning Spinner Flasks	0.324	0.541	0.500	\$ 132.10	5.00E+05	4.00E+06	4	1.62E+08	1.30E+09	0.284
Viable Cells		0.068	0.125	0.125	\$ 114.30	5.00E+05	4.00E+06	4	2.03E+07	1.62E+08	0.041
	Total:	\$ 632,538.80	1.00E+07	4.00E+06	0						

FIGURE A.1: Material Balance Excel Sheets.

Appendix B

Costing Summary Supplementary Information

Below is the Excel spreadsheet for the costing of our plant design.

	Unit	FOB	Capital Cost	Variable Cost	Annualized
Media		\$ 20,000.00	\$ 60,000.00	\$ 66,300.00	\$ 1,392,300.00
	Mixing Tank	\$ 20,000.00	\$ 60,000.00	\$ -	\$ -
	Sf-900 III SFM	\$ -	\$ -	\$ 66,300.00	\$ 1,392,300.00
Quality Control & Safety		\$ 31,800.00	\$ 95,400.00	\$ 1,460.00	\$ 3,260.00
	Beckman DU-640 Spectrophotometer	\$ 5,000.00	\$ 15,000.00	\$ -	\$ -
	Beckman Rectangular Open Top Cuvettes	\$ -	\$ -	\$ 275.00	\$ 1,375.00
	Tuttnerau 3870EA Large Capacity Fully Automatic Autoclave	\$ 15,000.00	\$ 45,000.00	\$ 1,150.00	\$ 1,150.00
	Series Large Capacity Distillers model 7000*12	\$ 8,000.00	\$ 24,000.00	\$ 35.00	\$ 735.00
	Milwaukee MW101 pH Tester	\$ 800.00	\$ 2,400.00	\$ -	\$ -
Utilities	oThermo Scientific HAAKE High-range portable viscometer	\$ 3,000.00	\$ 9,000.00	\$ -	\$ -
		\$ -	\$ -	\$ 575.95	\$ 12,095.00
	Electricity	\$ -	\$ -	\$ 380.95	\$ 8,000.00
	Oxygen	\$ -	\$ -	\$ 175.00	\$ 3,675.00
	Water	\$ -	\$ -	\$ 15.00	\$ 315.00
Waste Treatment	Sewage	\$ -	\$ -	\$ 5.00	\$ 105.00
		\$ 200,000.00	\$ 600,000.00	\$ 350.00	\$ 10,500.00
	Kill Tank	\$ 200,000.00	\$ 600,000.00	\$ -	\$ -
	Waste Management		\$ -	\$ 350.00	\$ 10,500.00
Seed Train & Production Reactor	Other?		\$ -	\$ -	\$ -
		\$ 640,000.00	\$ 1,920,000.00	\$ 15,538.80	\$ 326,314.80
	Corning 125 mL Flask	\$ -	\$ -	\$ 114.30	\$ 2,400.30
	Corning 500 mL Flask	\$ -	\$ -	\$ 132.10	\$ 2,774.10
	Corning 3 L Flask	\$ -	\$ -	\$ 272.50	\$ 5,722.50
	Corning36 L Flask	\$ -	\$ -	\$ 2,019.90	\$ 42,417.90
	Xcellerex XDR 200 L Single-Use Bioreactor	\$ 40,000.00	\$ 120,000.00	\$ 3,000.00	\$ 63,000.00
	Xcellerex XDR 2000 L Single-Use Bioreactor	\$ 300,000.00	\$ 900,000.00	\$ 5,000.00	\$ 105,000.00
Harvesting & Separating	Xcellerex XDR 2000 L Single-Use Bioreactor	\$ 300,000.00	\$ 900,000.00	\$ 5,000.00	\$ 105,000.00
		\$ 162,800.00	\$ 488,400.00	\$ 53,866.67	\$ 1,096,200.00
	Carr UniFuge Pilot	\$ 60,000.00	\$ 180,000.00	\$ 7,000.00	\$ 147,000.00
	Sartrou Maxicap size 3	\$ 2,800.00	\$ 8,400.00	\$ 3,200.00	\$ 67,200.00
	Chromatography	\$ 100,000.00	\$ 300,000.00	\$ 32,000.00	\$ 672,000.00
Inactivation	MiniKros Filter Modules	\$ -	\$ -	\$ 11,666.67	\$ 210,000.00
		\$ 20,000.00	\$ 60,000.00	\$ 18.00	\$ 540.00
	20 L Mixed Tank	\$ 20,000.00	\$ 60,000.00	\$ -	\$ -
Storage	Formaldehyde	\$ -	\$ -	\$ 18.00	\$ 540.00
		\$ 30,000.00	\$ 85,000.00	\$ 1,000.00	\$ 30,000.00
	Liquid Nitrogen	\$ 20,000.00	\$ 60,000.00	\$ -	\$ -
	PALL Allegro 2 D Biocontainer	\$ -	\$ -	\$ 1,000.00	\$ 30,000.00
TOTAL		\$ 1,084,600.00	\$ 3,248,800.00	\$ 139,091.42	\$ 2,870,669.80

FIGURE B.1: Costing Excel Sheets.

Appendix C

Economic Analysis Supplementary Information

On the following page is the Excel spreadsheet for the economic analysis of our plant design.

End of year	Investment			Yearly Cash Flow (\$1,000's)			Tax Allowance Earned in Previous Years			DCF Terms			
	Plant	Building	Working capital	Total	Production (1000s of doses)	Productio n cost	Sales revenue	Cash flow before tax	Plant	Building	Total	Cash flow after tax & grant	Cumulative cash flow
1	\$3,200.00	\$19,112.50	\$0.00	\$22,312.50	\$1,800.00	\$0.00	\$24,112.50	\$0.00	\$0.00	\$0.00	\$24,112.50	\$19,230.15	
2	\$0.00	\$0.00	\$0.00	\$0.00	12,500	\$3,575.00	\$0.00	\$3,575.00	\$0.00	\$0.00	\$0.00	\$3,575.00	\$27,687.50
3	\$0.00	\$0.00	\$0.00	\$2,000.00	25,000	\$4,550.00	\$11,000.00	\$4,450.00	\$480.00	\$1,435.63	\$1,435.63	\$4,450.00	\$23,237.50
4	\$0.00	\$0.00	\$0.00	\$0.00	37,500	\$5,525.00	\$16,500.00	\$10,975.00	\$408.00	\$955.63	\$1,363.63	\$0.00	\$10,975.00
5	\$0.00	\$0.00	\$0.00	\$0.00	50,000	\$6,500.00	\$22,000.00	\$15,500.00	\$346.80	\$955.63	\$1,302.43	\$9,672.58	\$12,262.50
6	\$0.00	\$0.00	\$0.00	\$0.00	50,000	\$6,500.00	\$22,000.00	\$15,500.00	\$294.78	\$955.63	\$1,250.41	\$14,249.60	\$2,190.84
7	\$0.00	\$0.00	\$0.00	\$0.00	50,000	\$6,500.00	\$22,000.00	\$15,500.00	\$250.56	\$955.63	\$1,206.19	\$14,293.81	\$3,227.53
8	\$0.00	\$0.00	\$0.00	\$0.00	50,000	\$6,500.00	\$22,000.00	\$15,500.00	\$212.98	\$955.63	\$1,168.60	\$14,331.40	\$3,237.55
9	\$0.00	\$0.00	\$0.00	\$0.00	50,000	\$6,500.00	\$22,000.00	\$15,500.00	\$181.03	\$955.63	\$1,136.66	\$14,363.34	\$3,246.06
10	\$0.00	\$0.00	\$0.00	\$2,000.00	50,000	\$6,500.00	\$22,000.00	\$17,500.00	\$153.88	\$955.63	\$1,109.50	\$14,390.50	\$3,259.45
11	\$741.17	\$0.00	\$0.00	\$741.17	-	\$0.00	\$0.00	\$741.17	\$130.80	\$955.63	\$1,086.42	\$16,413.58	\$2,976.50
												\$61,346.27	
												IRR:	25.39%

FIGURE C.1: Economic Analysis Excel Sheets.

Bibliography

- [1] Sang-Moo Kang, Jae-Min Song, Fu-Shi Quan, and Richard W. Compans. Influenza vaccines based on virus-like particles. *Virus research*, 143(2):140–146, Aug 2009. ISSN 1872-7492. doi: 10.1016/j.virusres.2009.04.005. URL <http://www.ncbi.nlm.nih.gov/pubmed/19374929>.
- [2] Mark Jit, Anthony T. Newall, and Philippe Beutels. Key issues for estimating the impact and cost-effectiveness of seasonal influenza vaccination strategies. *Human vaccines & immunotherapeutics*, 9(4):834–840, Apr 2013. ISSN 2164-554X. doi: 10.4161/hv.23637. URL <http://www.ncbi.nlm.nih.gov/pubmed/23357859>.
- [3] Robert B. Couch. Seasonal inactivated influenza virus vaccines. *Vaccine*, 26 Suppl 4:D5–D9, Sep 2008. ISSN 0264-410X. doi: 10.1016/j.vaccine.2008.05.076. URL <http://www.ncbi.nlm.nih.gov/pubmed/18602728>.
- [4] World Health Organization. Recommended composition of influenza virus vaccines for use in the 2013-14 northern hemisphere influenza season, . URL (3)http://www.who.int/influenza/vaccines/virus/recommendations/2013_14_north/en/.
- [5] World Health Organization. Influenza a(h1n1)pdm09 candidate vaccine viruses for vaccine development and production for the northern hemisphere 2013-14, .
- [6] World Health Organization. Influenza a(h3n2) candidate vaccine viruses and potency testing reagents for vaccine development and production for the northern hemisphere 2013-14, .
- [7] World Health Organization. Influenza b yamagata lineage candidate vaccine viruses and potency testing reagents for vaccine development and production for the northern hemisphere 2013-14, .
- [8] Health Industry Distributors Association. 2007-2008 influenza vaccine production and distribution: Market brief. URL http://www.preventinfluenza.org/HIDA_flubrief07-08.pdf.

- [9] Centers for Disease Control and Prevention (CDC). Prevention and control of seasonal influenza with vaccines. recommendations of the advisory committee on immunization practices- united states, 2013-2014. *MMWWR. Recommendations and reports: Morbidity and mortality weekly report. Recommendations and reports/Centers for Disease Control*, 62(RR-07):1–43, Sep 2013.
- [10] Novais J.L. Economic comparison between conventional and disposables-based technology for the production of biopharmaceuticals. *Biotechnology and Bioengineering*, 75(2):143–153, 2001.
- [11] Nell Greenfield-Boyce. New and old ways to make flu vaccines. URL <http://www.npr.org/templates/story/story.php?storyId=16105360>.
- [12] James T. Matthews. Egg-based production of influenza vaccine: 30 years of commercial experience. *The Bridge: Linking Engineering and Society*, 36(3):17–24, 2006.
- [13] Centers for Disease Control and Prevention (CDC). Cell-based flu vaccines. URL <http://www.cdc.gov/flu/protect/vaccine/cell-based.htm>.
- [14] Passport Health. Cell-based and egg-free flu vaccines, 2014. URL <http://www.passporthealthusa.com/vaccinations/cell-based-and-egg-free-flu-vaccines/>.
- [15] Robinson J and Bader B. Economics of influenza vaccine production using virus-like particles in insect cell culture. URL <http://www.novavax.com/download/File/Interphex.pdf>.
- [16] Downey W. Trends in biopharmaceutical contract manufacturing. *Chimica Oggi-Chemistry Today*, 31(1):19–24, Jan 2013.
- [17] Lowe A, Asaert W, Bachet P, Lotto L, and Holmes R. Contract manufacturing in life sciences: The need for an integrated approach. URL http://www.lodestonemc.com/files/pdf/WP_Contract_Manufacturing_in_Life_Sciences.pdf.
- [18] Baxter. Baxter’s biopharma solutions named ”best contract manufacturing organization” for third consecutive year at vaccine industry excellence awards. URL http://www.baxter.com/press_room/features/2012/vie_award.html.
- [19] Life Technologies. Sf-900 iii sfm - the reliable choice for insect cells, . URL <http://www.lifetechnologies.com/us/en/home/life-science/cell-culture/insect-cell-culture/insect-cell-culture-misc/sf-900-iii-sfm-the-reliable-choice-for-insect-cells.html>.

- [20] Beason B. Day 1: Biological buffers.
- [21] Life Technologies. Growth and maintenance of insect cell lines, .
- [22] Haitham Amen. Baculovirus and insect cell expression. URL http://www.academia.edu/1436672/Baculovirus_and_Insect_Cell_Expression.
- [23] Nitar Nwe, Qigai He, Sudarat Damrongwatanapokin, Qingyun Du, Ivanus Manopo, Yukol Limlamthong, Beau James Fenner, Lynn Spencer, and Jimmy Kwang. Expression of hemagglutinin protein from the avian influenza virus h5n1 in a baculovirus/insect cell system significantly enhanced by suspension culture. *BMC microbiology*, 6:16, Feb 2006. ISSN 1471-2180. doi: 10.1186/1471-2180-6-16. URL <http://www.ncbi.nlm.nih.gov/pubmed/16504108>.
- [24] Pneumatic Scale Angelus. Carr unifuge pilot. URL <http://www.psangelus.com/docs/design-group-library/psa-brochure-unifuge.pdf?sfvrsn=0>.
- [25] Sartorius Stedim Biotech. Sartopure gf plus midicaps and maxicaps. URL http://www.sartorius.com/fileadmin/fm-dam/sartorius_media/Bioprocess-Solutions/Filtration_Technologies/Prefiltration/Sartopure/Data_Sheets/Data_SartopureGF-Plus_MidiCaps_SPK2082-e.pdf.
- [26] GE Healthcare. Akta avant 150, . URL <http://www.gelifesciences.com/webapp/wcs/stores/servlet/productById/en/GELifeSciences/28976337>.
- [27] Spectrum Labs. Minikros sampler filter modules. URL <http://www.spectrumlabs.com/filtration/MiniKrosSampler.html>.
- [28] Sinclair A., Leveen L., Monge M., Lim J., and Cox S. The environmental impact of disposable technologies. *BioPharm*, 2008.
- [29] Reed Construction. Construction cost estimates for warehouse in chicago, illinois. URL <http://www.reedconstructiondata.com/rsmeans/models/warehouse/illinois/chicago/>.
- [30] Centers for Disease Control. Vaccine safety. URL <http://www.cdc.gov/vaccines/pubs/pinkbook/downloads/safety.pdf>.
- [31] Nelson K. Approaches for flexible manufacturing facilities in vaccine production. *BioPharm International Supplements*, pages s22–s28, Nov 2011.
- [32] OSHA. Osha directorate of training and education. URL <https://www.osha.gov/dte/>.

- [33] GE Healthcare Life Sciences. An environmental life cycle assessment comparison of single-use and conventional bioprocessing technology. URL http://www.gelifesciences.com/gehcls_images/GELS/Related%20Content/Files/1384357132198/litdoc29085317_20131114224350.pdf.
- [34] Rawlings B and Pora H. Environmental impact of single-use and reusable bioprocess systems. *BioProcess International*, pages 18–25, Feb 2009.
- [35] Aswath Damodaran. Tax rate data sets. URL <http://www.stern.nyu.edu/~adamodar/pc/datasets/taxrate.xls>.
- [36] GE Healthcare. New production methods and convertible systems to increase epidemic or pandemic surge capacity, . URL http://www.who.int/influenza_vaccines_plan/resources/loeillot.pdf.