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Manufacturing the next generation of vaccines: non-egg based platform for influenza vaccine

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Manufacturing the Next Generation of Vaccines:
Non-egg Based Platform for Influenza Vaccine

An Undergraduate Honors College Thesis

in the

Department of Chemical Engineering

College of Engineering

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by

Andrew B. Price

December 13, 2013

This thesis is approved.

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Jamie Hestekin

For my honors thesis project, I formed a group with two other chemical engineering students, Nathan Havens and Chris Walls, to complete the AIChE 2014 student design competition problem dealing with designing a vaccine production facility that uses a non-egg based expression platform. My specific roles in the project focused heavily on researching, selecting, and pricing appropriate equipment for the process and compiling the majority of the written report. My partners in the project helped tremendously with supplying supplementary materials (economics, calculations, figures, tables, PFD, etc.) as I compiled the material for the written report.

Andrew Price

Letter of Transmittal

Nathan Havens, Andrew Price, Christopher Walls
Ralph E. Martin Department of Chemical Engineering
University of Arkansas-Fayetteville

American Institute of Chemical Engineers
120 Wall St.
New York, NY 10005

Dear reader,

The enclosed contains design information for a manufacturing facility that will produce influenza vaccine using an Sf9 insect cell expression platform rather than the traditional egg-based platform. Advantages to this approach include faster times to market and increased production. This report shows that the process is economically viable and indeed very profitable. The facility is designed to produce 60 million trivalent vaccine doses per year which covers over one third of the market.

Consider this report only a preliminary design. More research on market conditions and the production process is to be done to be ready for a full-scale launch. However, this report does show that there is exciting and profitable opportunity for a company on the leading edge of these new vaccine production methodologies.

Manufacturing the Next Generation of Vaccines:

Non-egg Based Platforms for Influenza Vaccine

AIChE Student Design Competition 2014

By

Nathan Havens

Andrew Price

Christopher Walls

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Fayetteville, AR

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I: Abstract

This report explores the design of an influenza vaccine manufacturing facility using a non-egg based expression platform. The current standard procedure in flu vaccine production is to grow the virus in bird eggs. However, recently there is evidence that supports production by other expression platforms. The Sf9 insect cell is considered in this report. Facility design including cell culture media formulation through product storage for shipment and final formulation is considered based on the use of an existing facility. The new design also employs the use of disposable equipment such as bioreactors and purification unit operations. These disposables help reduce production time and are an economically viable option.

Market trends are analyzed, and it is concluded that production capabilities for this proposed facility should be about 60 million doses per year to accommodate one third of the market and be able to account for potential epidemics. At this production, revenue is approximately $391 million per year. Capital costs are very low due to the use of the existing facility, and manufacturing costs are also very reasonable even with the use of disposable equipment. Based on a 10 year project lifetime, the NPV for the project is approximately $460 million. The project is economically robust when considering various factors such as fluctuating dosage selling price, dosage demand, and manufacturing costs.

A major concern for the profitability of this project is if the market will accept large quantities of vaccine produced by new methods that are not historically tested and proven to work. More research should conducted to determine how the market will react to such changes.

The next step in the design process is to conduct laboratory and pilot-scale tests to determine more exact cell and virus growth curves for the seed train and the bioreactors. Once this information is collected, more precise designs can be formulated. Based on the considerations in this report, the production of influenza vaccine using the Sf9 expression platform is viable both economically and in its ability to meet market demands in a timely manner. Companies on the leading edge of this development will have a great deal to gain.

II: Introduction

Many new challenges and opportunities have arisen in the field of vaccine production in the twenty-first century. Increased product demand, concerns about product quality, and a greater focus on reducing environmental impact are a few of the challenges facing the biopharmaceutical industry [13]. However, with each of these challenges comes an opportunity for ingenuity and development within the industry. One area of increased focus within the industry has been that of developing non-egg based platforms for the production of influenza vaccines. The traditional expression platform for influenza vaccine development is bird eggs. This process has proven to be safe, reliable, and adaptable to World Health Organization (WHO) yearly standards. However, the egg-based platform also comes with risks including a potential sudden decrease in supply due to a bird flu outbreak and patient allergies to eggs and feathers [7].

Recent developments in equipment and technology have made Chinese hamster ovary and insect cell expression systems realistic options for influenza vaccine production platforms [5,4]. These cell-based expression systems may offer increased production and faster times to market for the vaccines people need. The major drawback to these platforms is that they have not been used extensively in large-scale production like the egg-based platform. However, the initial investment of time, research, and financial recourses may prove to be very profitable for companies on the leading edge of this development.

This report contains preliminary design information for an existing production facility that is being transitioned to produce a trivalent influenza vaccine using the Sf9 insect cell expression system [4]. The purpose of this design report is to provide enough information for management to determine if this is a suitable production method to pursue. The design provides a general scope of the process from the preparation of cell culture media to final product purification. Final formulation and product packaging are mentioned but not considered in depth. Equipment specifications and process economics are also discussed. A brief discussion of traditional clean-in-place equipment and newer disposable equipment is also contained in this report.

III: Process Description

The Process Flow Diagram (PFD) and overall material balance for the process are shown in Figure 1 below. The process consists of cell culture media preparation and scale-up seed train, virus inoculation and growth in production bioreactors, virus recovery and purification, and product storage. The product would then be shipped to the final formulation group for formulation and packaging.

Cell Culture Media Formulation

There are several options for appropriate cell culture media. One option that suits the needs for this process is “Grace’s insect media” developed by Life Technologies™. The media is prepared onsite by mixing media powder with water in M-101 according to the procedure provided by Life Technologies™[9]. The mixer uses disposable pre-sterilized bags each containing 40 L of culture media.

Seed Train

To begin the seed train, 1 L of culture media is added to a flask along with a vial of 1×10^7 viable Sf9 insect cells. The scale up process takes approximately 160 hours per batch. The cells are expanded

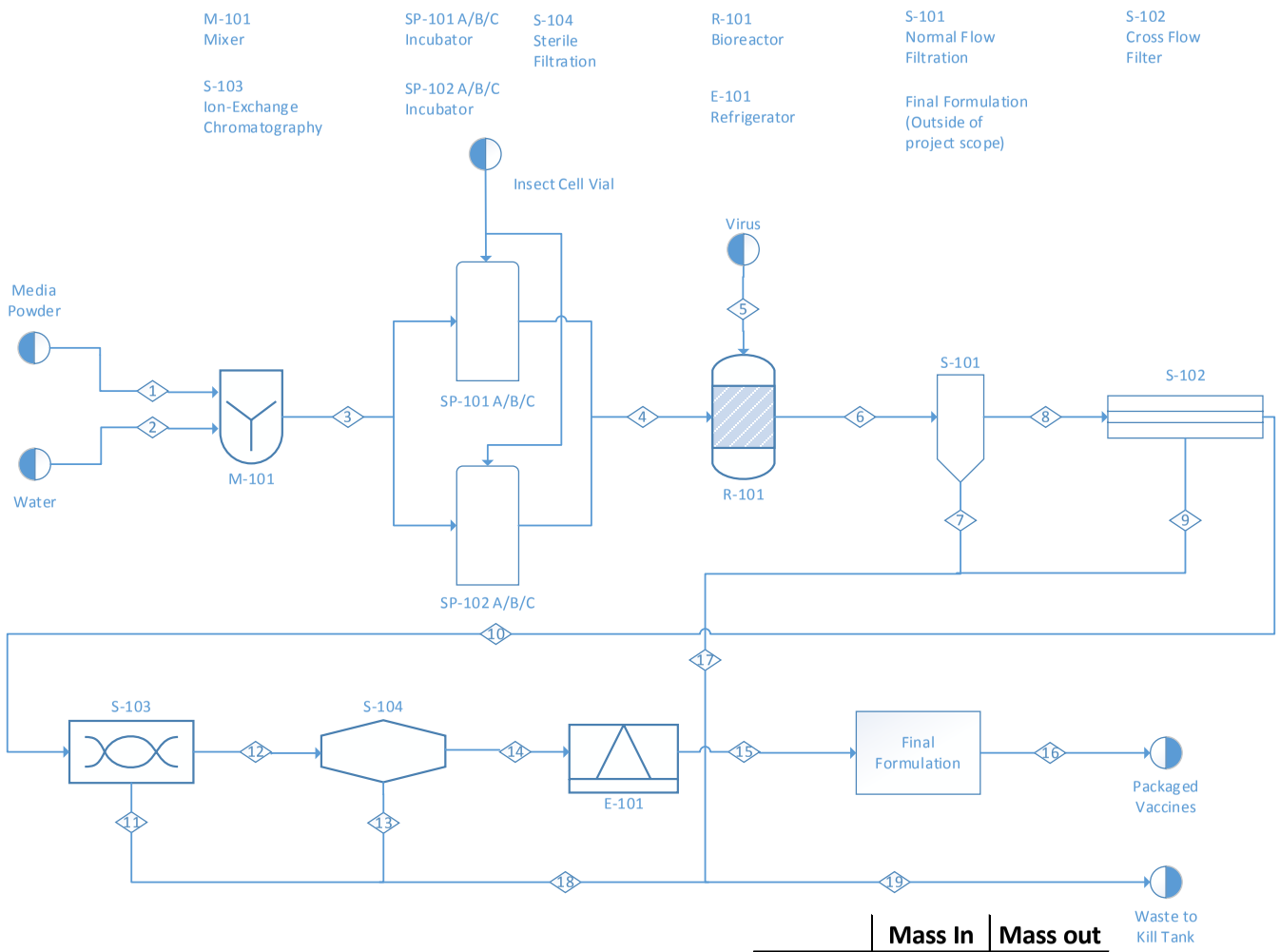

Figure 1: Process flow diagram and overall material balance for a single monovalent batch of the vaccine production process

	Mass In	Mass out
Water	40kg	----
Product	----	0.3kg
Waste	----	39.7kg

by batch wise addition into larger volumes of media over time up to the final volume of 40 L. Over this time period, the cells are incubated to provide maximum growth rate. Once the final scale-up is completed, the mixture is ready for the production bioreactor.

Production Bioreactor

The Sf9 culture broth is added to the bioreactor and inoculated with the particular strand of influenza virus being used in the batch. Although the vaccine being produced is trivalent, only a single moiety of the vaccine can be produced in each batch. The bioreactor (R-101) uses disposable 50 L bags to contain each 40 L batch. Each batch remains in the bioreactor for 50 hours. For the purposes of this design it was assumed that the Sf9 cells follow a typical growth curve as shown below in Figure 2 [1].

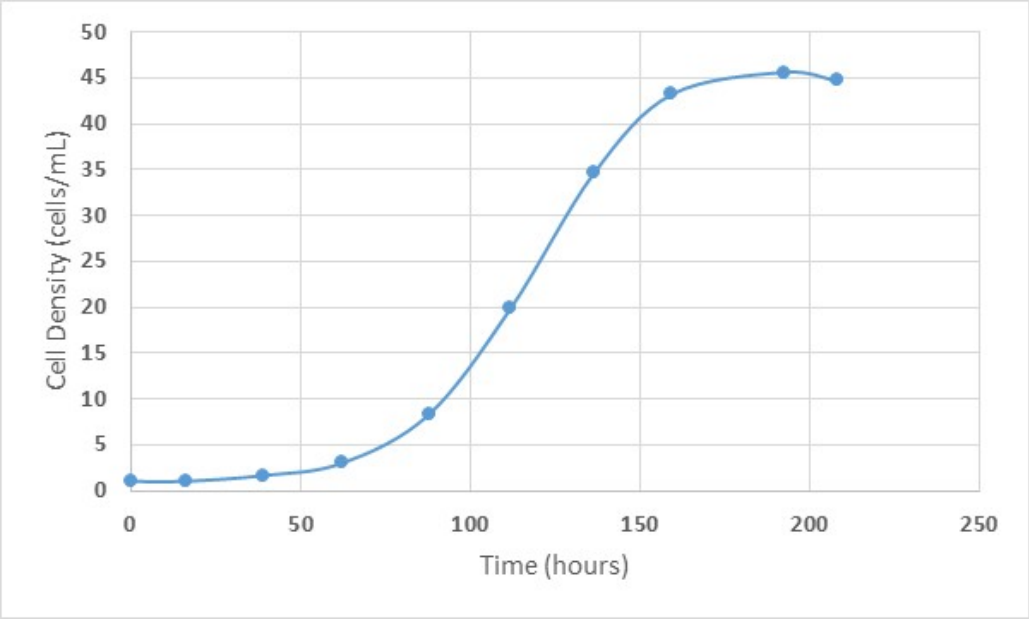

Figure 2: Typical cell density growth curve for Sf9 insect cells in a batch culture [1]

The first 160 hours represent scale-up in the seed train. Inoculation occurs at the 160 hour point. The next 50 hours account for virus production in the bioreactor. The broth is removed from the bioreactor at 210 hours. Again, this setup is based on a typical growth curve for Sf9 cells. If this production method is pursued, it would be quite necessary to employ laboratory and pilot scale operations to determine growth rates in the specific equipment used for this process as well as optimum conditions for the seed train scale-up and virus production in the bioreactor.

Recovery, Purification, and Inactivation of Product

Three unit operations are used for recovery and purification of the product. Normal-flow filtration (S-101) is first used to remove the biomass from the product. Cross-flow filtration is then used to begin the purification process. Anion exchange chromatography (S-103) is used to complete the purification process. This method is widely accepted in the vaccine production industry and recommended specifically by GE Healthcare™[3].

For the inactivation process, it is necessary to select a method that safely inactivates the virus without destroying the product. The method used in this design is sterile filtration (S-104). Each of the

purification and inactivation techniques employ disposable cartridges rather than traditional clean-in-place vessels.

Storage and Shipment

The final product from each batch will be freeze dried for proper storage according to CDC recommendations [12]. The product will be stored in its individual moieties. At this point in the process, the moiety products will be ready for shipment to the final formulation group as the market demands. Final formulation of the trivalent vaccine and packaging for patients will occur at this point. This final step in the process is outside the scope of this report and can be handled by the professionals in the formulation group as demand requires.

Production Waste

This report investigates using a current facility for a new method of production. Therefore, it is assumed that the current sewer system and "kill tank" systems can be employed for the safe and reliable handling of all production waste from this process.

Sample Analysis

Sample analysis and quantification is extremely important to maintain product quality. Equipment designed for this purpose may be available on site. If not, the Biacore T200 manufactured by GE Healthcare™ is a viable option.

Batch Scheduling

The rate-limiting steps in the production process are the seed train and the bioreactors. Therefore, a general scheduling procedure can be outlined from these two steps in the process. A sample batch schedule for the seed train and bioreactor is shown below in Figure 3. The time required to produce the total required amount of a single moiety is approximately 35 days (840 hours). The time required to produce all of the required moieties is approximately 105 days.

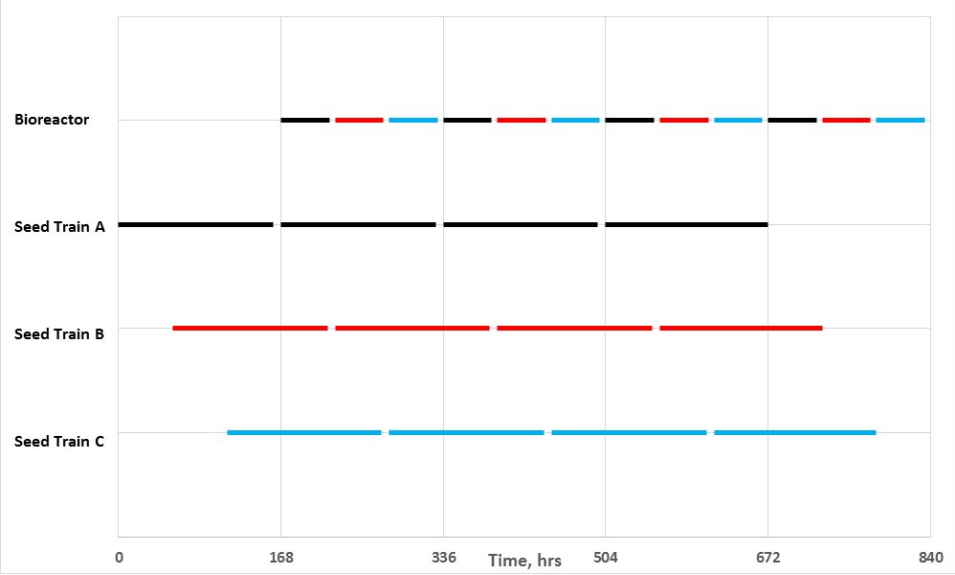

Figure 3: Sample seed train and bioreactor batch schedule for the complete production cycle of a single moiety

The time required for the seed train for each batch is 160 hours. In addition, it is assumed that 8 hours of cleaning, sterilizing, and preparing for the new batch will be required for each batch since traditional clean-in-place vessels are used in this part of the process. The time required in the bioreactor is 50 hours. With a 6 hour time period between each batch, the time in the bioreactor is one third of the time required in the seed train. Therefore, production time can be optimized by having three independent seed trains running at once. This is reflected by seed trains A, B, and C in Figure 3. The sequence for each batch in the bioreactor is also shown in Figure 3. The three independent seed trains are not indicated in the PFD in Figure 1 because this diagram is designed on a "per batch" basis.

Disposable Equipment vs. Clean-in-Place Equipment

There has been a recent shift in biopharmaceutical manufacturing towards disposable equipment [8]. Advantages of this technology include reduced capital investments, faster facility construction and launch, and reductions in time and costs for equipment sterilizations. However, traditional clean-in-place equipment has been proven effective for many years and requires a significantly lower consumables budget for manufacturing. The process design in this report employs both traditional and disposable equipment. Clean-in-place vessels are used in the seed train. Most other parts of the process use disposable equipment for the reasons outlined above. As this new equipment becomes more popular and widespread, production costs will likely decrease which will help eliminate the major drawback of a much greater consumables budget.

IV: Utility Requirements

One major advantage of using disposable equipment is the elimination of steam required for cleaning and sterilizing process vessels. The major utility requirements for this facility would be water and power. The power requirements for the facility are not estimated in this report because the cost is assumed to be insignificant at this stage in the design process. Further investigation into utility costs can be done if plans for this facility advance in the future. Water requirements are estimated and accounted for in the economics section of this report.

V: Equipment Specifications

The equipment specification sheet is shown on the next page in Figure 4. This specification sheet represents the necessary purchases for transitioning the facility to a non-egg based expression platform. Other equipment is required for the process but is assumed to be on site. This includes sample analysis equipment, "kill tank" systems for pre-sewage treatment, and miscellaneous equipment. Other equipment required for purchase that is not included in the PFD or equipment specifications sheet is a number of small flasks for the seed train scale-up procedure.

The bioreactors and separation equipment were all chosen from GE Healthcare's line of disposable vaccine production equipment. Purchasing the majority of the equipment from a single manufacturer will improve compatibility and customer service should any issues arise.

VI: Equipment Cost and Fixed Capital Investment Summary

Since the facility for this production process already exists, the Fixed Capital Investment (FCI) for this project will consist of only new capital equipment purchases. A summary of the capital costs for this

Mixers
M-101
WAVE mixer manufactured by GE
Dimensions: 0.50 m x 0.38 m x 0.17 m
Maximum operating volume: 35 L
Use M*Bag
Volume: 35 L
Material: Plastic
Reactors
R-101
WAVE bioreactor manufactured by GE
Stainless steel
Dimensions: 1.85 m x 1.09 m x 1.12 m
Use Cellbag Bioreactors
Volume 50 L
Material: Plastic
Special Purpose Equipment
SP-101 A/B/C, 102 A/B/C*
Incubator manurfactured by Cole-Parmer
Stainless steel
Temperature range: 5 C to 105 C
Capacity: 24.79 cuft
Chamber dimensions: 39.5 in x 52.6 in x 20.6 in
*There are 6 total incubators employed simultaneously, but only 2 are used on a per batch basis
Refrigeration
E-101
HM LY series pharmaceutical vacuum freezing dryer
Manufactured by HM Pharmachine
Condenser temperature: -70 C
Cooling rate: 20 to 40 C per 45 min
220 V

Separators
S-101
Ulta Prime GF manufactured by GE
Normal flow filtration
Area: 0.56 square meters
Pore size: 0.6 micron
Glass microfiber filtration membrane
10 inch capsule length
S-102
AKTAcrossflow manufactured by GE
Cross flow filtration
Dimensions: 0.62 m x 0.40 m x 0.65 m
Use AXM/AXH ultrafiltration cross flow cartridges
Material: polysulfone
Membrane area: 42 square cm
S-103
AKTA Ready Flow Kit manufactured by GE
Ion (anion) exchange chromatography
Use Capto Q chromatography cartridges
Bed dimensions: 80 mm x 200 mm
Highly cross-linked agarose matrix
S-104
Ulta Pure SG
Sterile Filtration
Area: 0.047 square meters
Pore size: 0.2 micron
Polyethersulphone membrane material

Figure 4: Equipment specification sheet

process is given on the next page in Table 1. The prices are based on online prices or direct quotes from the manufacturer.

The total FCI for the process is $1 million. This number is obviously low for a pharmaceutical facility, but it should be considered that the vast majority of the FCI would have been incurred in the purchase of land and building of the actual facility. The most significant costs are the freeze dryer, sample analysis equipment, and bioreactor.

VII: Manufacturing Costs

A summary for the annual manufacturing costs for the process is given on the next page in Table 2. The total annual Cost of Manufacturing (COM) is $2.8 million. The largest piece of that total by far is the operating labor costs due to the extremely precise nature of the process. Prices for Grace's insect media and disposable equipment were found online. The price of water was set to the standard value. Operating labor costs were calculated as outlined in Turton's process design text [11].

Table 1: Summary of the FCI for the new vaccine production process

Item	Total Quantity	Unit Cost	Total Cost
WAVE Mixer	1	$ 9,889.00	$ 9,889.00
Spinner Flasks (6L)	30	$ 950.00	$ 28,500.00
Incubator	6	$ 8,900.00	$ 53,400.00
WAVE bioreactor	1	$ 190,190.00	$ 190,190.00
Cross Flow Filtration Machine	1	$ 81,102.00	$ 81,102.00
Chromatography add-on for CFF machine	1	$ 8,100.00	$ 8,100.00
Sample analysis machine	1	$ 367,400.00	$ 367,400.00
Freeze dryer	1	$ 200,000.00	$ 200,000.00
Freezer	2	$ 9,833.00	$ 19,666.00
Total			**$ 958,247.00**

Table 2: Summary of annual manufacturing costs for the new vaccine production process

Item	Total Quantity	Unit Cost	Total Cost
Grace's Insect Media	$ 108.00	$ 87.00	$ 9,396.00
WAVE Mixer M*Bags	$ 36.00	$ 122.00	$ 4,392.00
Reactor cellbags (50L)	$ 36.00	$ 233.00	$ 8,388.00
Normal Flow Filters	$ 36.00	$ 224.00	$ 8,064.00
Cross Flow Filtration Cartridges	$ 72.00	$ 216.00	$ 15,552.00
Chromatography cartridges	$ 36.00	$ 11,832.00	$ 425,952.00
Sterile filtration cartridges	$ 36.00	$ 388.00	$ 13,968.00
Water for Injection (L)	$ 1,800.00	$ 1.00	$ 1,800.00
Operating labor			$ 2,018,857.00
Sterilization			$ 347,000.00
Total			**$ 2,853,369.00**

VIII: Market Conditions and Revenue Analysis

Demand for influenza vaccine has increased sharply over the past decade as shown in Figure 5 [6]. The CDC reports that in the 2013-2014 flu season about 145 million vaccine doses will be produced [10]. If this trend continues for the next flu season, it is reasonable to assume that about 150 million doses of vaccine will be produced in the 2014-2015 season. To support one third of the market, a major pharmaceutical company could produce about 50 million doses of vaccine. To account for a potential epidemic, production capabilities should be at 60 million doses. This assumption of 60 million doses produced will be carried through the rest of the economic analysis in this report. The calculation method for determining the number of batches to process to account for 60 million doses is shown in Appendix A.

The base revenue for this facility is determined simply by the number of doses produced multiplied by a constant selling price per dose. According to the CDC, the flu vaccine price per dose is about $10.85 [2]. Assuming a wholesale rate of 60% of that market price, the price per dose of vaccine

sold would be $6.51. Using this constant price and a value of 60 million doses, yearly revenue would be $391 million.

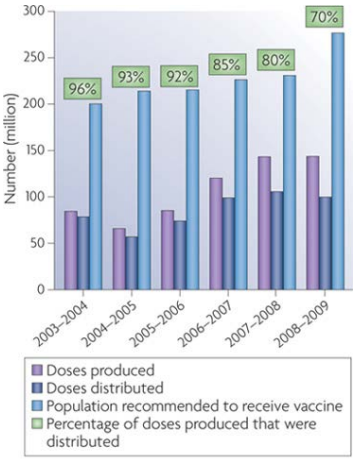

Figure 5: Market trend of influenza vaccine supply and distribution since 2003 [6]

IX: Economics Analysis

The analysis presented here is based on a 10 year project lifetime with one year of setup required. An internal rate of return (IRR) of 50% is used along with a tax rate of 40%. A working capital of 30% of the FCI is used. The economic analysis for this base case scenario is presented below in Table 3. Years 5-8 were removed from the chart for the purposes of this report. The full economic analysis chart is available in Appendix B. The analysis folows the method outlined in Turton's process design text [11].

Table 3: Base case economic analysis for the proposed production facility

End of Year (k)	0	1	2	3	4	9	10	11
Investment	$ (958,247.00)	$ -	$ -	$ -	$ -	$ -	$ -	
Depreciation (dk)	$ -	$ 191,649.40	$ 306,639.04	$ 183,983.42	$ 110,390.05	$ -	$ -	$ -
FCIL-∑dk	$ -	$ 766,597.60	$ 459,958.56	$ 275,975.14	$ 165,585.08	$ 958,247.00	$ 958,247.00	$ 958,247.00
Revenue	$ -	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00
COM	$ -	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00
(R-COM-dk)*(1-t)+dk	$ -	$ 232,724,638.36	$ 232,770,634.22	$ 232,721,571.97	$ 232,692,134.62	$ 232,647,978.60	$ 232,647,978.60	$ 232,647,978.60
Cash Flow (CF)	$ (958,247.00)	$ 232,724,638.36	$ 232,770,634.22	$ 232,721,571.97	$ 232,692,134.62	$ 232,647,978.60	$ 232,647,978.60	$ 232,647,978.60
Cumulative CF	$ (958,247.00)	$ 231,766,391.36	$ 464,537,025.58	$ 697,258,597.55	$ 929,950,732.17	$ 2,093,256,859.20	$ 2,325,904,837.80	$ 2,558,552,816.40
Discounted CF	$ (958,247.00)	$ 155,149,758.91	$ 103,453,615.21	$ 68,954,539.84	$ 45,963,878.44	$ 6,051,707.82	$ 4,034,471.88	$ 2,689,647.92
Cumulative Discounted CF	$ (958,247.00)	$ 154,191,511.91	$ 257,645,127.11	$ 326,599,666.96	$ 372,563,545.40	$ 452,378,195.34	$ 456,412,667.22	$ 459,102,315.14

The net present value (NPV) for the project is $459 million after a 10 year lifetime. This is quite impressive, but again it must be considered that the capital costs are considerably low due to the use of an existing facility. Even so, it is clear that the new production process outlined in this report is quite profitable.

Sensitivity Analyses

It is also important to consider the proposed project's sensitivity to market conditions as well as to the assumptions made in this report about the design. Figure 6 below shows the project's economic sensitivity to three key factors: reduction in product selling price, reduction in the number of doses sold, and increased cost of manufacturing. A reduction in selling price has the expected effects with a 50% decrease in price resulting in about a 50% decrease in NPV. However, it is unlikely that such a price

fluctuation would occur in this market, so this will not be much of a factor on the project's profitability. The number of doses sold could fluctuate somewhat if demand did not increase yearly as expected. Again, though, this is a very stable market with a stable client base, and the number of doses sold is unlikely to drop by a significant amount. The key point to consider here is that while the market is stable for the current production process, it is yet unclear if the market will react favorably to a new production method or if the market will be slow to accept a new production method as safe and reliable. This could potentially lower the amount of doses that this project would be able to move in the market and thus reduce profit.

The most important factor to examine is the cost of manufacturing. Some assumptions have been made in this report in regards to the manufacturing process especially in determining how many batches of product will be needed per year. The validity of these assumptions has not been tested experimentally. This leaves uncertainty in the manufacturing cost calculations. Figure 6 shows that the process is extremely robust in this area, though. A 50% increase in manufacturing costs has almost no effect on the NPV. Even if costs were greatly understated, this should not affect the profitability of the project.

The new production method seems to be economically profitable and robust. As long as the market is thought to be open to new technologies and methods of production, project profitability should not be a cause for concern for the Sf9 production platform.

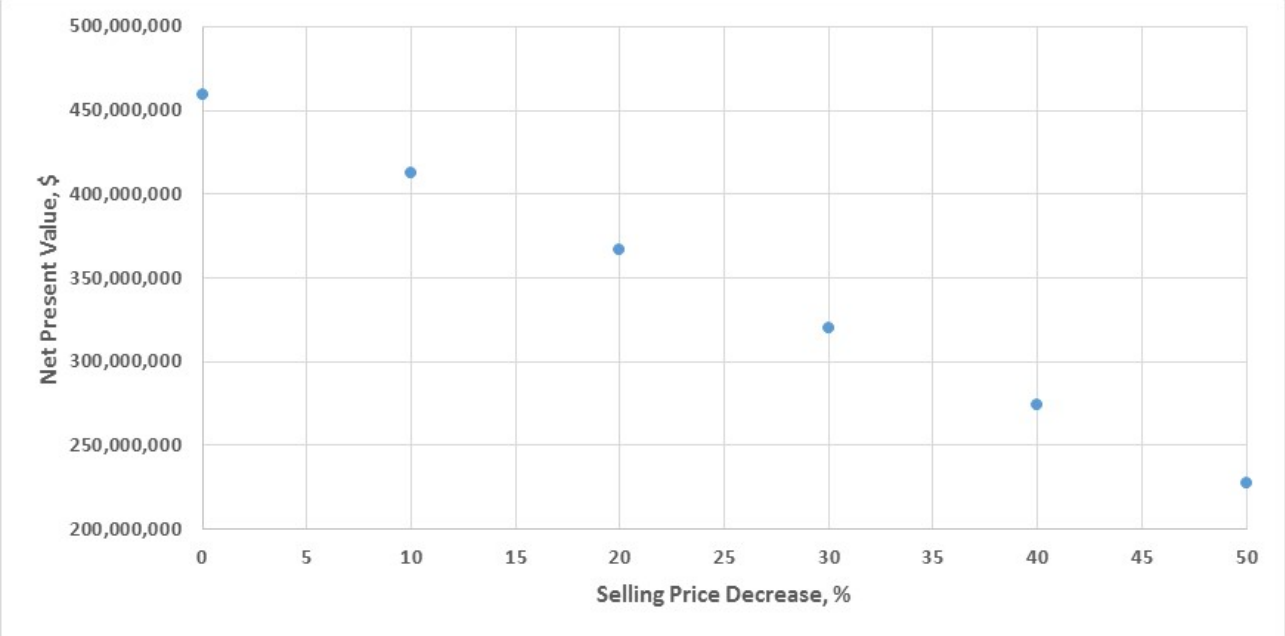

Figure 6a

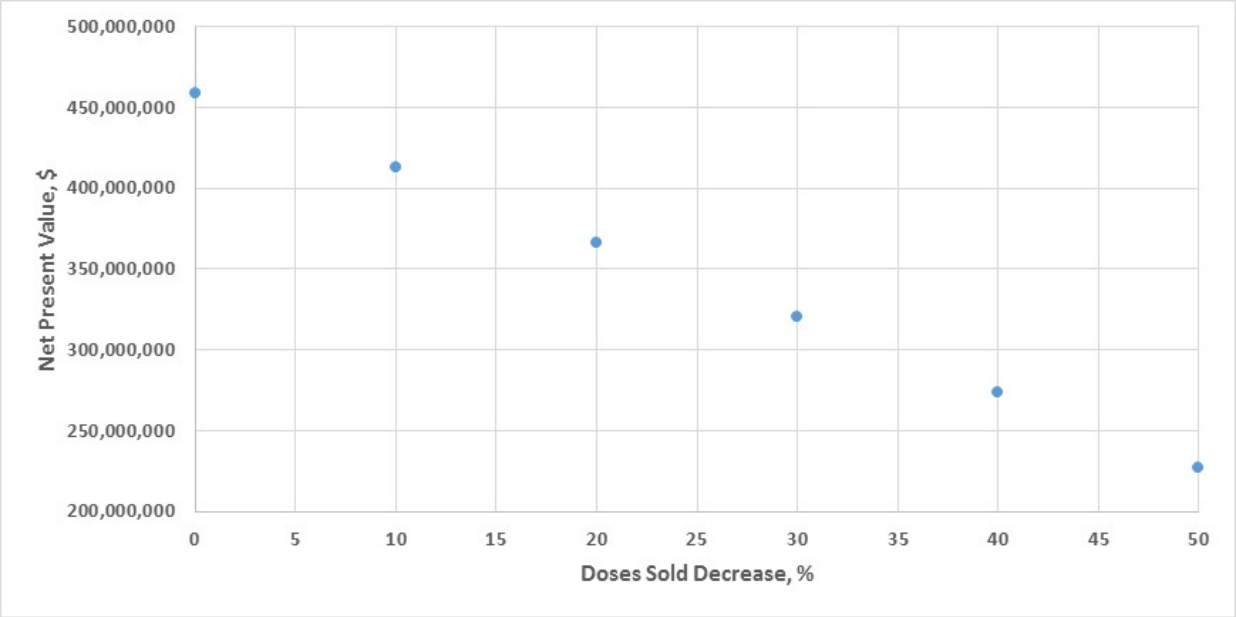

Figure 6b

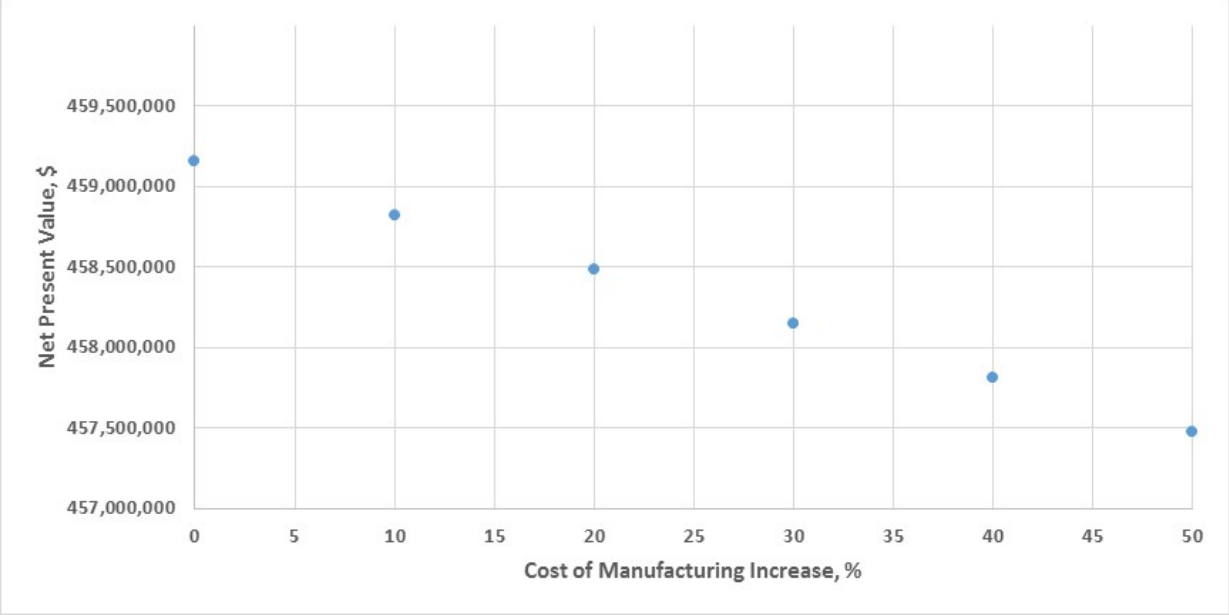

Figure 6c

Figure 6: Sensitivity of project NPV to a) price of vaccine on the market, b) number of doses sold, and c) manufacturing costs

X: Safety, Health, and Environmental Considerations

There are major health and safety concerns when dealing with vaccine production. However, most of these concerns will not be new to the production facility simply because the expression platform changes. Current operating procedures will be sufficient to handle most of the changes that would occur. The cell culture media is chemically defined, so the facility will be animal free. The production team will require additional training and development for using the new single-use

disposable equipment. Disposal procedures will need to be established to ensure safe disposal of all single-use equipment. Existing waste management systems may prove sufficient for the new process. However, preliminary tests should be conducted to ensure safe and reliable waste management. Facility sterilization is still budgeted into the design economics as a safety measure even though most of the equipment used in this design would be pre-sterilized single-use disposables.

XI: Conclusions and Recommendations

1. Influenza vaccine production by non-egg based platforms is a reasonable design consideration based on its ability to meet and exceed market demand in a reasonable time period and its economic viability.
2. Production of 60 million doses of influenza vaccine using an Sf9 insect cell expression platform can be completed in three to four months economically in a facility similar to the design proposed in this report.
3. Disposable equipment offers a viable alternative to traditional clean-in-place equipment. It is easy to use, can decrease production times, and was not shown to have a major negative economic impact on the design. The design herein employs disposable reactors, mixers, and filtration equipment.
4. Considering recent market trends in the supply and distribution of the influenza vaccine, production capabilities should be 60 million doses per year to control one third of the market and be prepared to meet market needs during an outbreak.
5. Capital costs in this report are quite low due to the use of an existing facility. This should be taken into account when considering the overall profitability of the project.
6. The economic analysis for the project is favorable with a NPV potential of $460 million for a 10 year project lifetime at a 50% IRR.
7. The project is economically robust in regards to selling price and manufacturing costs.
8. More research should be done on how the market will react to a new production method that is not “time-tested.” Even if production rate is not a problem, moving the vaccine in the market may be an issue using this new technology.
9. Few new safety considerations arise with the transition to an Sf9 expression platform. The production team should be trained on any new disposable equipment employed in the process.
10. Existing waste treatment protocols should be tested with the new production method to see if new equipment or protocol is required.
11. The next step in the design process is to conduct laboratory and pilot-scale tests to determine more exact cell growth and virus growth profiles for more detailed designs in the future.

XII: Acknowledgements

A great deal of gratitude is owed to the Ralph E. Martin Department of Chemical Engineering at the University of Arkansas for the continued support of its students in every possible way. It is also owed to Dr. Robert Beitle for providing his students with the opportunity to participate in this contest. Finally, thanks to AIChE for its support of chemical engineering students and for providing the contest problem each year.

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XIV: Appendix A – Calculation Methodologies for Production

The density of the broth exiting the reactor was given as 1.06 g/mL. The assumption was made that 1.00 g/mL could be accounted for by water, and that 0.02 g/mL could be accounted for by the cells and the virus in the broth. The radius of an Sf9 cell is estimated at 16 µm before inoculation and 20 µm when the virus is present in the cell [1]. The increase in volume is attributed to the virus, and a percentage of the amount of virus in the cells is calculated to be 0.488. Assuming that 80% of the virus produced in the reactor is active, the virus density is calculated by multiplying the overall density by the infection rate and the percent virus to give a virus density of 0.0078 g/mL. Knowing that 60 µg of each moiety is required per dose, the doses per mL of broth is calculated to be 130. The total dose production allows for the calculation of total broth production. The volume of broth per batch is also known which leads to the calculation of 12 batches per moiety for a total batch count of 36 for the trivalent vaccine. The calculation is outlined in Table A-1 below.

Table A-1: Calculation methodology for determining the total number of batches required to meet production requirements

	Amount	Unit
Total broth density	1.06	g/mL
Density of cells and virus	0.02	g/mL
Sf9 cell radius pre-virus	16	um
Sf9 cell radius inoculated	20	um
Sf9 cell volume pre virus	17148.59	um^3
Sf9 cell volume inoculated	33493.33	um^3
Volume of virus	16344.75	um^3
Percentage virus	0.488	---
Active virus fraction	0.8	---
Active virus density	0.007808	g/mL
Moeity required for each dose	6.00E-05	g/mL
Doses per mL of broth	130.13	dose/mL
Total doses needed	6.00E+07	dose
Broth needed	4.61E+05	mL
Volume broth per batch	40000	mL
Batches required (each moeity)	11.53	batch

In addition, since each seed train and bioreactor cycle take 210 hours combined and there are 12 batches per moiety and 3 available seed train batches at once, the total time for a single moiety production is 840 hours (35 days).

XV: Appendix B - Complete Economics Analysis Table

Table B-1: Complete economics analysis table

End of Year (k)	0	1	2	3	4	5
Investment	$ (958,247.00)	$ -	$ -	$ -	$ -	$ -
Depreciation (dk)	$ -	$ 191,649.40	$ 306,639.04	$ 183,983.42	$ 110,390.05	$ 110,390.05
FCI_L-∑dk	$ -	$ 766,597.60	$ 459,958.56	$ 275,975.14	$ 165,585.08	$ 55,195.03
Revenue	$ -	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00
COM	$ -	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00
(R-COM-dk)*(1-t)+dk	$ -	$ 232,724,638.36	$ 232,770,634.22	$ 232,721,571.97	$ 232,692,134.62	$ 232,692,134.62
Cash Flow (CF)	$ (958,247.00)	$ 232,724,638.36	$ 232,770,634.22	$ 232,721,571.97	$ 232,692,134.62	$ 232,692,134.62
Cumulative CF	$ (958,247.00)	$ 231,766,391.36	$ 464,537,025.58	$ 697,258,597.55	$ 929,950,732.17	$ 1,162,642,866.79
Discounted CF	$ (958,247.00)	$ 155,149,758.91	$ 103,453,615.21	$ 68,954,539.84	$ 45,963,878.44	$ 30,642,585.63
Cumulative Discounted CF	$ (958,247.00)	$ 154,191,511.91	$ 257,645,127.11	$ 326,599,666.96	$ 372,563,545.40	$ 403,206,131.03

6	7	8	9	10	11
$ -	$ -	$ -	$ -	$ -	
$ 55,195.03	$ -	$ -	$ -	$ -	$ -
$ (0.00)	$ (0.00)	$ (0.00)	$ 958,247.00	$ 958,247.00	$ 958,247.00
$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00	$ 390,600,000.00
$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00	$ 2,853,369.00
$ 232,670,056.61	$ 232,647,978.60	$ 232,647,978.60	$ 232,647,978.60	$ 232,647,978.60	$ 232,647,978.60
$ 232,670,056.61	$ 232,647,978.60	$ 232,647,978.60	$ 232,647,978.60	$ 232,647,978.60	$ 232,647,978.60
$ 1,395,312,923.40	$ 1,627,960,902.00	$ 1,860,608,880.60	$ 2,093,256,859.20	$ 2,325,904,837.80	$ 2,558,552,816.40
$ 20,426,452.16	$ 13,616,342.60	$ 9,077,561.73	$ 6,051,707.82	$ 4,034,471.88	$ 2,689,647.92
$ 423,632,583.19	$ 437,248,925.79	$ 446,326,487.52	$ 452,378,195.34	$ 456,412,667.22	$ 459,102,315.14