MEMORANDUM

To: Sarah Ewing
AIChE Student Programs
From: Engineering Design Team
Date: March 9th, 2016
Subject: Letter of Transmittal

Enclosed is the report for the *Cell Therapy for Spinal Cord Injuries: Commercial Manufacturing Facility* to be submitted to the 2016 AIChE Student Design Competition. The project was to develop a stem cell manufacturing facility for the production of these cells to be used in the treatment of spinal cord injuries.

In this report, a manufacturing facility is designed with the ability to readily scale-up production to meet market demands. This is achieved by having up to four bio-reactor trains per module and the option to operate each bio-reactor between high and low production rates.

This facility was designed to be in compliance with current good manufacturing practices and current good tissue practices as set by the United States Food and Drug Administration. This is to ensure the health and safety of not only those individuals working at the facility but also the patients who receive the cell therapy from this company.

Please be aware that this report is concise. It is understood that management has limited time, and so every attempt was made to efficiently communicate the design process in a clear fashion without extraneous information.

This report is the preliminary design, economic analysis, and subsequent recommendation on how to proceed.

Cell Therapy for Spinal Cord Injuries: Commercial Manufacturing Facility

FOR THE

AIChE 2016 Student Design Competition

Executive Summary

This project was undertaken to provide a preliminary design of a facility that could manufacture stem cells for treatment of spinal cord injuries. The result of this design is a module facility that is capable of meeting the IRR of 50% at reasonable treatment prices even with low production.

It is recommended that management move this preliminary design forwarded to detailed design as quickly as possible. It is also recommended that a facet of the detailed design is a pilot test to validate that the number of treatments produced per batch matches the expectation set by the literature.

The facility is a module concept that allows for varied treatment production rates per bio-reactor train. It is also developed to accommodate up to three more bio-reactor trains per module with a slight increase in capital investment for each added train.

Table 1: Treatment Price Summary

	Production Type	Annual Treaments	Treatment Price	Percent Price Reduction
Scenario 1	Low Production, 1 Train	1,485	$ 2,466	-
Scenario 2	High Production, 1 Train	4,471	$ 1,005	59%
Scenario 3	High Production, 4 Train	17,882	$ 508	79%

Table 1 shows three different operational scenarios for the facility. Scenario 1 is a single bio-reactor train operating in a module at low production. This is the fewest amount of treatments that the facility would expect to produce. Scenario 2 takes the single bio-reactor train in the module and increases it to high production by reducing the amount of downtime between each manufacturing campaign. The amount of treatments produced is more than tripled and the price per treatment has dropped significantly. Scenario 3 showcases the facility operating at maximum capacity with four bio-reactor trains in the module at high production. It provides more than 12 times the original amount of annual treatments and at nearly 80% less than the initial treatment cost.

Extensive research has been done to ensure that the design is as accurate as possible to what should be expected in a manufacturing scale-up. Additionally, the sensitivity analysis done with a Monte Carlo simulation shows a stable economic projection in which small discrepancies cause little impact on the price per treatment 75% of the time. In 95% of the cases the treatment price did not exceed twice the initial price.

Furthermore, the facility has been designed to follow cGMP and cGTP as set by the FDA. This will ensure the health of the workers in the facility and the safety of the patients who receive these treatments.

There are no major health, safety, economic or operational issues in moving forward with this design.

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List of Tables and Figures

Introduction

This project was initiated by Dr. Robert Beitle, of the Ralph E. Martin Department of Chemical Engineering at the University of Arkansas. The project was started on February 8th, 2016.

The goal of this project is to design a manufacturing facility for the production of stem cells to be used in the treatment of spinal cord injuries. This facility is being constructed by a midsized company focusing on cell therapy technologies. The standalone assumption has been made for economic analysis of the project due to the size of the company and the absence of other projects that may have produced taxable revenue which could be reduced by losses incurred from this project.

Adult stem cells differentiated into specialized neural progenitor cells (NPCs) are a potential cell therapy for spinal cord injuries[1]. While adult stem cells that could be used for spinal cord injuries are naturally occurring in the human body, they are almost exclusively found in the brain[2]. This would require an expensive and invasive surgery to harvest a limited amount of cells to be used for treatment. In lieu of this option, NPCs can be differentiated from either human embryonic stem cells (hESC) or adult stem cells called induced pluripotent stem cells (iPSC). As the hESC program is behind the iPSC program (and significantly more contentious on an ethical level – which provides funding and availability problems), this facility is based on using iPSC technology. Therefore, it is assumed that the 1×10^5 vial of adult stem cells contains adult stem cells that have been induced into a pluripotent state.

The facility will be able to receive these cryogenically frozen iPSCs, thaw them, grow them into a larger quantity, differentiate them into the desired neural stem cells, purify the cells of incorrectly differentiated cells, separate them into the appropriate amount of cells required for one treatment and prepare them for shipping to off-site packaging. The facility also has several other components that are required for the process. This includes: storage on site for growth media and differentiation media, a quality control lab in order to ensure each batch is created successfully and is safe for use, storage of the finished product, and a disposal system to neutralize all wastes.

The process was designed to limit the necessity of human interaction while also providing an economically priced product. This reduces the risk of exposure to the workers and contamination of the cells by the workers.

When constructing this facility, it was determined that a modular design would be the most beneficial while also allowing for operational flexibility. This design allows for high and low production rates of up to four bio-reactor trains to be run in parallel. This provides anywhere from 1,485 to 17,882 treatments per year per module. This flexibility is built in to allow the

[1] (Ogawa et al., 2002)
[2] (Zhao, Deng, & Gage, 2008)

company to meet market demand as it changes. The current United States market is nearly 250,000 injured patients with the addition of nearly 12,000 patients a year. The module design provides the company the option to easily increase production and eventually manufacture the product in larger markets such as Europe or Asia.

Technical Discussion

Design Basis

One of the most significant decisions made in the design process is that iPSCs behave very similarly to hESCs[3,4]. The design utilizes coefficients, growth conditions, and constraints for hESCs and applies them to iPSCs. This seems reasonable as an iPSC is supposed to be an adult stem cell reprogrammed to behave as if it were back into its previous embryonic state.

The market justification for this facility was to meet the demand in the United States market for spinal cord injury repair with a long term goal of treating patients in Europe and beyond. The current United States market has approximately 250,000 people with spinal cord injuries and an additional 12,000 people are injured every year.

The facility is currently developed to produce 1,485 to 17,882 treatments per module per year depending on the number of reactor trains. This treatment amount could allow for a steady reduction in injured individuals in the United States. In order to provide the operational flexibility to scale up production into larger markets the facility is designed in a modular fashion in which a small, standard facility is created with excess capacity so that more reactor trains could be located in the same module. The module can have up to three more reactor trains added to the original reactor train, bringing the total to four reactor trains, in order to increase production while only incurring a slight increase in capital investment and a near proportional increase in operating costs.

Additionally, each reactor train can operate within a range between high and low production. Production levels can be changed by reducing the down time of the differentiation reactor (BR-3).

[3] (Hu et al., 2010)
[4] (Takahashi et al., 2007)

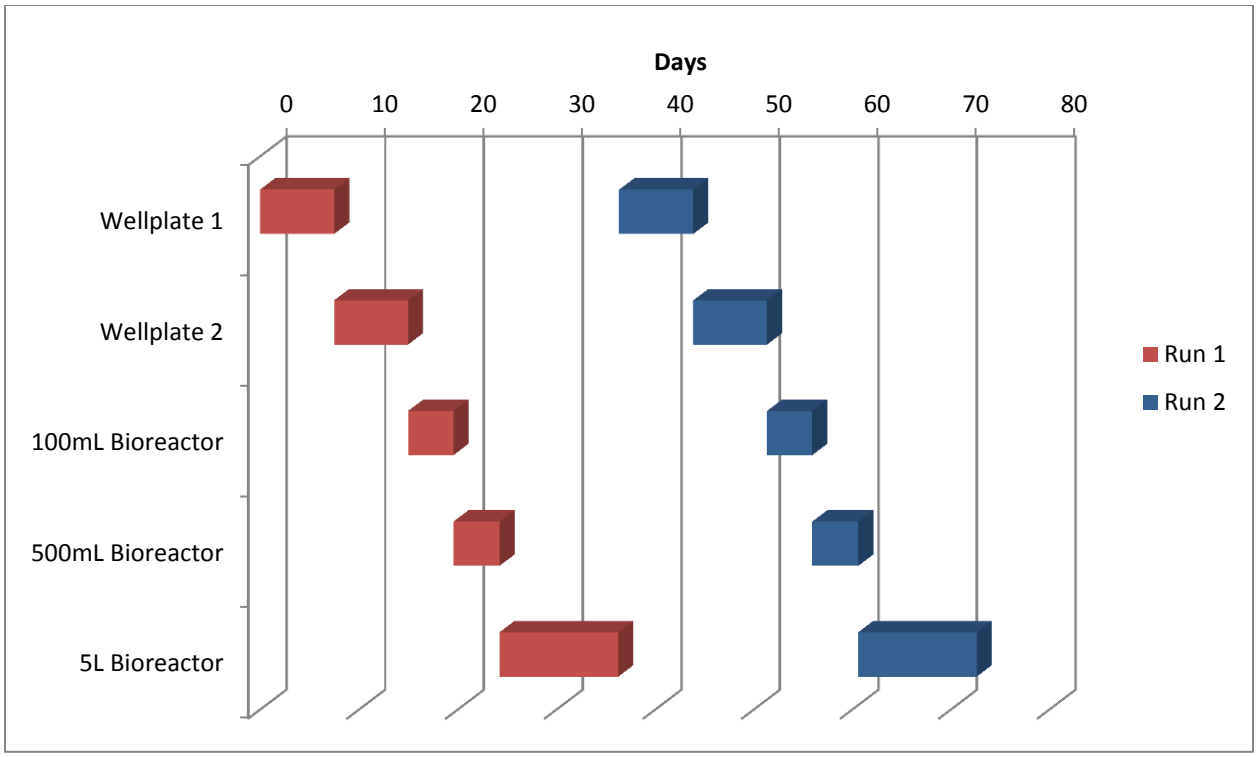

Figure 1: Low Production Gantt Chart

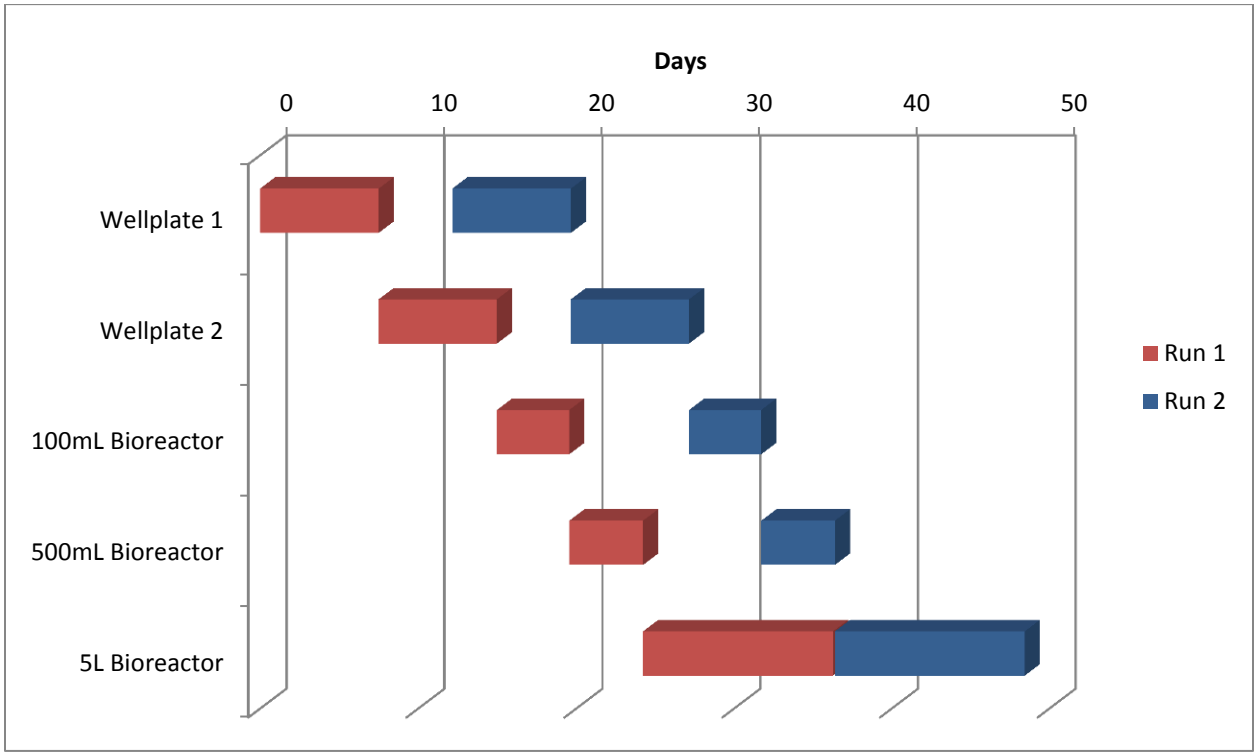

Figure 2: High Production Gantt Chart

At high production levels the down time of the BR-3 is reduced to just enough time to allow for cleaning and turn around (just under 3 hours) as can be seen in Figure 2: High Production Gantt Chart**.** This design focused on providing both high and low production level estimates in order to well define the boundaries of the process. As there are no known technical difficulties with operating the reactor trains at some intermediate production level this would be feasible if desired by management. The exact operating conditions and costs associated with the selected intermediate production level would fall within the operating conditions and costs defined between high and low production.

Furthermore, the module design allows for construction of the module in optimal locations. A capital intensive facility constructed in a single location (say California or New York) would be challenged to provide treatments that require lengthy shipment distances cross-country let alone overseas. This is undesirable due to the delicate nature of the cells. While the cells should have a reasonably long shelf-life when frozen, the farther they travel the greater the probability becomes that the cells are damaged, contaminated, or killed due to the shipment being handled incorrectly. Small, reasonably priced modules can be built it multiple locations across the country or overseas to reduce shipment distances.

Lastly, because the product is a biological one, various restrictions and rules may be imposed by different governments and trade organizations on products that are to be imported into their domains. Though stem cell treatments are an emerging technology and are not as heavily regulated as some of the more developed biological product areas such as organ donations, this regulation may occur in the future as stem cell treatments become more readily available[5]. Therefore the ability to construct new facilities that are self-contained within these regulatory areas and have the appropriate amount of modules to adequately meet each regions production requirements is economically attractive and operationally more efficient.

Clearly, it is desirable to have the ability to locate facilities close to the markets they will service. It is easier, cheaper, and less risky to ensure the safe shipment of the initial adult stem cells required to begin the process than it is to ensure the safe shipment of all the treatments leaving the facility across equally long distances.

Design Philosophy

This project is focused around the nascent field of large scale production of adult stem cells. Thus, the majority of the information contained in this report comes from peer reviewed published scientific papers. This provides the best justification for using certain criteria to set expectations. It is understood that while some variability may occur in scaling up the process

[5] (Shimazono, 2007)

from a laboratory setting to a manufacturing setting the expectation is that the numbers provided in the literature will track well on larger scales[6].

While bio-manufacturing facilities have become well developed over the past few decades they tend to deal with more rugged cells such as bacteria. This facility, however, deals with adult stem cells which require notably more careful treatment than the majority of other bio-processes. Careful treatment can be seen in the time intensive pouring and pipetting of cellular solutions by hand as opposed to the traditional pumping of cellular solutions throughout the process. Furthermore, the traditional fermenter reactors used to grow large amounts of cells are unable to be used as adult stem cells require an adherent growth environment; such as a manifold, a wall, or a microcarrier. Lastly, the cells must be maintained within strict environmental conditions for optimal growth and proper differentiation[7].

These special processing requirements for the cells set constraints on several aspects of the design. Chief among these is a constraint on the volume of the reactors. As the reactors become larger, the ability to maintain consistent environments and the well mixed assumption for the cellular solution becomes difficult without increasing the rotational speed of the reactors. Increasing rotational speed is problematic. As the rotational speed of the reactors exceeds 100 rotations per minute (RPM), cells begin to die due to the sheer forces[8]. Therefore, as reactors become larger it becomes more difficult to prevent thermal or nutrient gradients from occurring within the reactor. The increase in volume also makes managing the amount of total liquid in the process more difficult. Lastly, due to the exponential nature of cell growth, small increases in volume can result in massive increases in total cell treatments produced. This causes operational difficulties with managing large number of treatments produced in a single batch in a timely manner.

Another constraint was to reduce the chances of contamination while also minimizing the financial impact should contamination ever occur. Chances for contamination are minimized by following Current Good Manufacturing Practices (cGMP) as laid out by the Federal Drug Administration (FDA). These practices are in place to protect facility personnel and to ensure that a safe product is provided to patients. Health and safety were considered throughout the design of this facility, more detailed discussion about those considerations can be found in the Health and Safety section.

[6] (Fernandes et al., 2009)
[7] (dos Santos, Andrade, da Silva, & Cabral, 2013)
[8] (Baghbaderani et al., 2008)

Process Description

For a graphical representation of this process please consult Figure 3: Process Flow Diagram for Stem Cell Facility. For a better understanding of the layout of the clean room please consult Figure 4: Clean Room Top Down Layout. These are located at the end of this section.

The entire manufacturing process occurs within an ISO Class 3 clean room. This is to limit the potential for contamination by airborne pathogens or particulates. Technicians will perform all tasks using aseptic technique.

All steps in the process should follow aseptic technique and procedures should be explicit to prevent miscommunication or mislabeling of cells in the process. The oxygen levels when growing the cells will be 30% of air saturation which results in 8% dissolved oxygen[9]. The oxygen levels when differentiating cells need to be at 4% air saturation which results in 1% dissolved oxygen[10]. Agitation of the cellular solution occurs in the spinner flask and onward.

The process starts with thawing the 1×10^5 iPSCs. The thawing protocol should follow the industry standard, something akin to the one outlined in ThermoFisher Scientifics protocol was considered in designing the facility[11].

After the cells have been thawed, they will be plated into Well Plate 1 (WP-1). The well plate is then placed within the incubator (I-1) to maintain temperature. The well plate is coated with an adherent matrix that allows for cell fixation and growth within the plate. The cells are in the well plate for 7.5 days before they reach the maximum cell density, are cleaved from the well plate by the addition of trypsin, and are then transferred to the next system in the bio-reactor train.

The next system in the bio-reactor train is Well Plate 2 (WP-2). This well plate is also located within I-1 to maintain temperature. The well plate is also coated with an adherent matrix that allows for cell fixation and growth within the plate. The cells are in the well plate for 7.47 days before they reach the maximum cell density, are cleaved from the well plate by the addition of trypsin, and are then transferred to the next system in the bio-reactor train.

The next system in the bio-reactor train is Bio-reactor 1 (BR-1) which is a 100 mL spinner flask. It will operate at 60 RPM and be stirred with a magnetic stir bar. The flask will sit on a magnetic stir plate with a heating element to maintain temperature. Microcarriers are added to the flask to provide attachment points and structure for the cells. The cells are in this flask for 4.57 days before they reach the maximum cell density and are then transferred to the next system in the bio-reactor train.

[9] (Serra et al., 2010)
[10] (Rodrigues, Fernandes, Diogo, da Silva, & Cabral, 2011)
[11] (ThermoFisher, 2016)

The next system in the bio-reactor train is Bio-reactor 2 (BR-2) which is a 500 mL bio-reactor. It will operate at 60 RPM and is stirred by impeller in the reactor. This reactor will have internal controls for temperature, dissolved oxygen, and pH monitoring. More microcarriers will be added to this reactor to provide more growing area for the cells[12]. The cells are in this reactor for 4.66 days before they reach maximum cell density.

At this point in the process the adult stem cells have been grown from the starting amount of 1 X 10^5 cells to 1.23 X 10^9 cells. Notice that up until this point in the process the cells have simply been grown to larger and larger quantities. During the next step in the bio-reactor system the cells will continue to grow and begin to differentiate due to changes in the environment. These environmental pressures are differences in the growth factors and other components contained in the differentiation media that are not present in the growth media as well as different oxygen concentrations[13]. Ensuring proper conditions are maintained will result in the highest yield of stem cells differentiated into NPCs.

When the cells are finished growing in BR-2 they are collected and cleaved from the microcarriers through the addition of trypsin to the solution. The cells are passed through a 40 micron filter to remove the microcarriers before the cells are moved to the next system in the bio-reactor train.

The next system in the bio-reactor train is Bio-reactor 3 (BR-3) which is the differentiation reactor. This reactor uses two different kinds of media in the next two steps of the process. The first step is introducing the filtered cells from BR-2 into the system with the addition of neural induction media to begin the differentiation of the stem cells into NPCs. This step takes six days to allow formation of neural rosettes, which are aggregate embryoid bodies[14]. BR-3 like BR-2 will be able to control environmental factors such temperatures, dissolved oxygen, and pH.

After the formation of the neural rosettes the cellular solution will be filtered and placed back into BR-3. The filtration process here is a double filter that has a 400 micron filter and a 100 micron filter. The double filter allows for everything larger than 400 microns - neural rosette formations that grew to larger and will have necrotic cells at the center - and smaller than 100 microns -neural stem cells that did not form rosettes, damaged adult stem cells, and stem cells that differentiated into undesirable forms - to be removed from the cellular solution leaving neural rosettes that are in the desired range of 100 to 400 microns in the filter to be replaced into BR-3[15].

Upon being replaced into BR-3 the filtered neural rosettes will stay in the reactor for another 6 days. During this time the media will be changed from the neural induction media to rosette

[12] (Bardy et al., 2013)
[13] (Technologies, 2011)
[14] (Technologies, 2011)
[15] (Technologies, 2015)

selection media which will break the neural rosettes down and allowing the newly differentiated NPCs to separate from themselves[16].

At the conclusion of the second set of six days in BR-3 the purified NPCs are removed from the reactor, excess media is removed from the cells (through a combination of decanting and centrifugation), the cells are re-suspended in freezing media, and then 2 mL of the solution is aliquoted into cryovials which are packed into a thermal regulating container that is placed within a refrigerator (F-3) operating at-50°C. The packed cryovials will cool at a rate of 1°/min until reaching -50°C. After the cells have reached -50°C they are moved to a nitrogen freezer (F-4) to be cooled down to -196°C to await pickup[17].

Waste disposal is handled in two parts. The first is liquid wastes that are generated daily from the removal of depleted media from the bioreactors and is placed in an autoclave (A-1) to be heated up to 121°C for 30 minutes before it is neutralized, if need be, and disposed down the drain. The second is solid wastes that are generated from general lab consumables coming into contact with cellular material. These wastes are either soaked for 30 minutes in 70% ethanol or placed within the in-lab UV cabinet before they are disposed of into the trash.

Stream Summary Tables and Process Flow Diagram

The stream summary table as seen in Table 2: **Stream Summary Table** on the next page provides information about the streams in the process flow diagram (PFD). In order to perform the mass balances in Table 4: **Media Mass Balance** and Table 5: **Cell Mass Balance**the calculations used the assumptions listed in the Technical Details and Design Practices section. It should be noted that these are an order of magnitude analysis based on the best available numbers from published literature. While the mass balance does not close because of unknown rates of production, exact cell densities, and exact media densities, the cell balance does close; this can be seen in Table 3: **Cell Balance**. The cell balance closes around the system and around each bio-reactor within the system.

The assumed values behind the mass balance with all included values can be found in Appendix A.

After Figure 3: Process Flow Diagram for Stem Cell Facility, a top down layout of the facility has been provided in Figure 4: Clean Room Top Down Layout to help understand positioning within the clean room. All manufacturing equipment is included and to scale.

Energy calculations were done in order to determine the amount of electricity required for utility costing. These calculations can be found in Appendix A: Energy Calculations.

[16] (Technologies, 2011)
[17] (Kielberg, 2010)

Table 2: Stream Summary Table

Stream	1	2	3	4	5	6	7	8	9	10	11	12
Number of Cells	1.00E+05	1.00E+05	1.52E+06	2.28E+07	1.49E+08	1.23E+09	9.80E+08	1.96E+09	1.57E+09	2.98E+09	2.98E+09	2.98E+09
Temperature (°C)	-130	37	37	37	37	37	37	37	37	37	-50	-195
Cell Mass (g)	0.0001	0.0001	0.00152	0.0228	0.149	1.23	0.98	1.96	1.57	2.98	2.98	2.98
Media volume (mL)	1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2	2	2
Heat Capacity (J/K)	3.99	0.00	0.00	0.02	0.16	1.29	1.03	2.06	1.65	11.11	11.11	11.11

Table 3: Cell Balance

Stream Transfer	1 to 2	2 to 3	3 to 4	4 to 5	5 to 6	6 to 7	7 to 8	8 to 9	9 to 10	10 to 11	11 to 12
Time Growing	-	7.5	7.47	4.57	4.66	-	6	-	6	-	-
Cells Grown	-	1500000	22480000	152200000	1.076E+09	-	9.8E+08	-	1.57E+09	-	-
Cells Lost	-	80000	1200000	26250000	245000000	-	3.92E+08	-	1.57E+08	-	-

Table 4: Media Mass Balance

Location	Container volume (mL)	Media volume added (mL/batch)	Media mass added (g/batch)	Glucose consumed (g/batch)	Glutamine consumed (g/batch)	Lactate produced (g/batch)	Ammonium produced (g/batch)	Sum of metabolites (g/batch)	Mass of media removed (g/batch)
Well plate 1	2	17.0	17.3	0.00	0.00	0.00	0.00	0.00	17.3
Well plate 2	30	254	259	-0.02	0.00	0.00	0.00	-0.02	259
Bioreactor 1	50	279	284	-0.09	-0.01	0.01	0.00	-0.09	284
Bioreactor 2	350	1982	2021	-0.66	-0.06	0.06	0.01	-0.65	2021
Rosette Formation	4000	28000	28560	-2.75	-0.25	0.25	0.03	-2.72	28557
Rosette Dissolution	5000	35000	35700	-4.41	-0.40	0.40	0.05	-4.36	35696

Table 5: Cell Mass Balance

Location	Biomass Produced (g/batch)	Number of cells produced	Calculated cell mass (g/batch)	Difference	% Difference
Well plate 1	1.42E-03	1.50E+06	1.50E-03	-8.35E-05	-5.89%
Well plate 2	2.12E-02	2.25E+07	2.25E-02	-1.25E-03	-5.89%
Bioreactor 1	9.21E-02	1.52E+08	1.52E-01	-6.01E-02	-65.24%
Bioreactor 2	6.51E-01	1.08E+09	1.08E+00	-4.25E-01	-65.36%
Rosette Formation	2.72E+00	9.80E+08	9.80E-01	1.74E+00	64.02%
Rosette Dissolution	4.36E+00	1.57E+09	1.57E+00	2.79E+00	64.02%

WB-1
10L Heated water bath

WP-1
2mL Well plate

WP-2
5mL 6 well plate

BR-1
100mL Stirred flask bioreactor

BR-2
500mL Stirred tank bioreactor

BR-3
5000mL Stirred tank bioreactor

UV-1
UV sterilization cabinet

A-1
19L Autoclave kill tank

F-3
-50°C Freezer

F-4
Liquid nitrogen chilled cryo-freezer

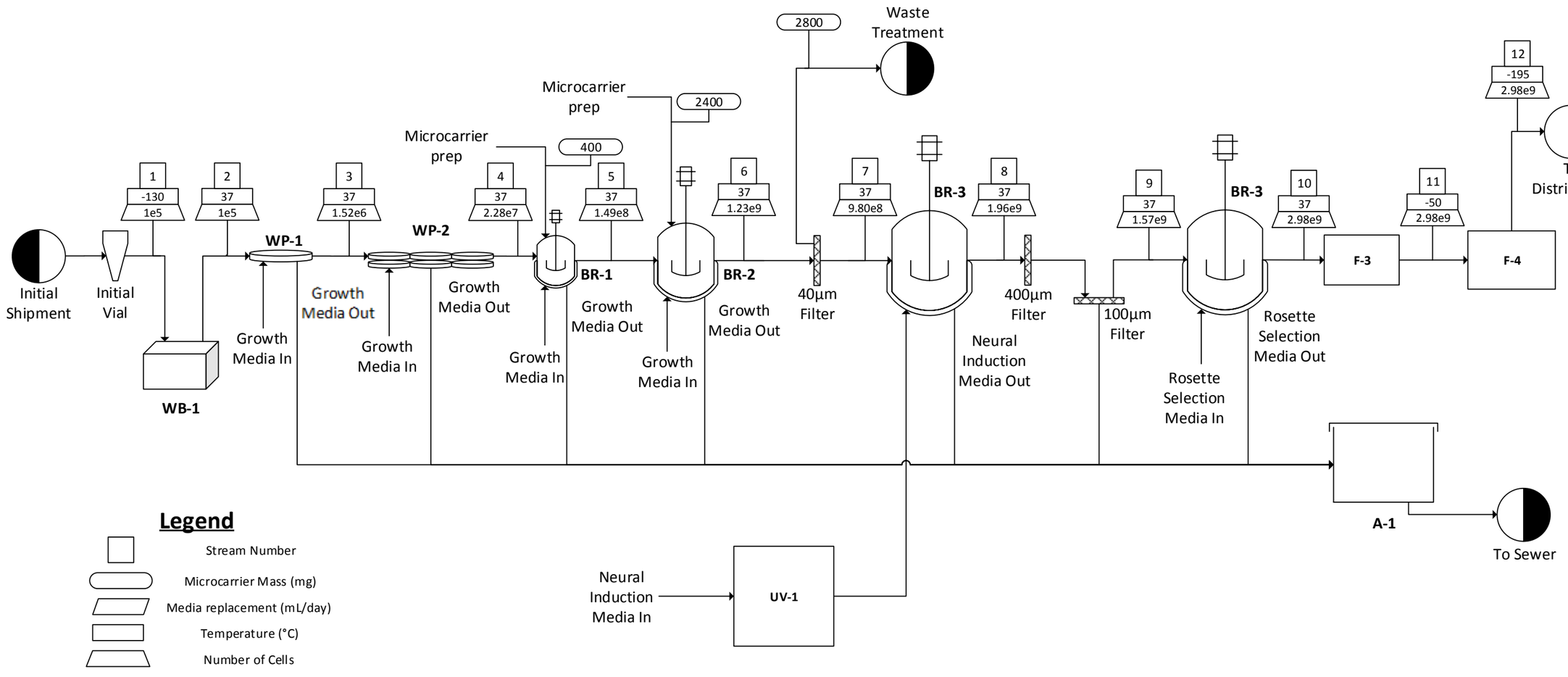

Figure 3: Process Flow Diagram for Stem Cell Facility

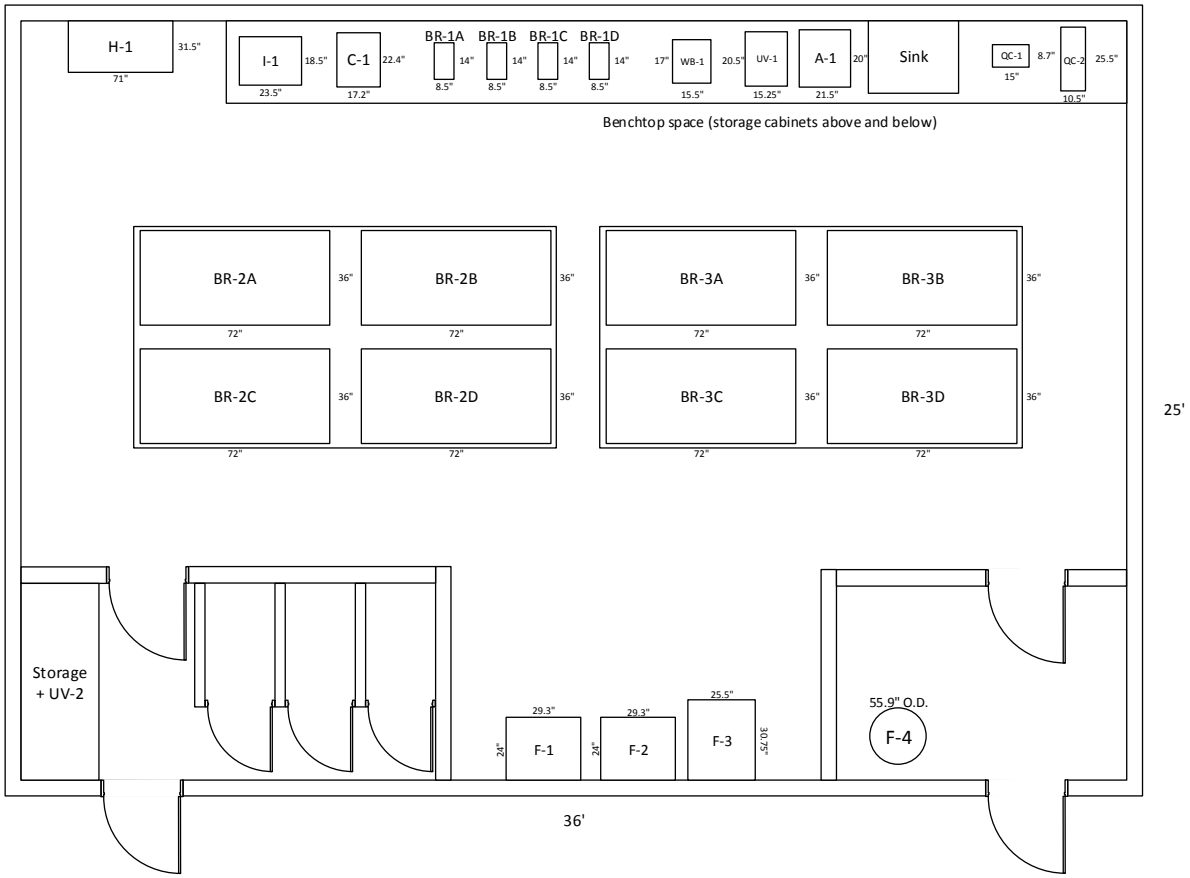

Figure 4: Clean Room Top Down Layout

Technical Issues and Design Practices

Key Assumptions

Several assumptions were made in this design project. These are outlined below and were used for calculations unless otherwise stated.

Cells:

1. The beginning amount of cells is 1 X 10^5 cells/batch
2. The beginning cells are induced pluripotent stem cells
3. The minimum cell density for seeding a well plate is 5 X 10^4 cells/mL[18]
4. The maximum cell density for a well plate is 8 x 10^5 cells/mL*
5. The minimum cell density for seeding a reactor is 1.25 X 10^5 cells/mL*
6. The maximum cell density for a reactor is 3.5 X 10^6 cells/mL*
7. The doubling time for two dimensional growth (well plates) of iPSCs is 33 hours*
8. The doubling time for three dimensional growth (microcarriers) is 21 hours*
9. The doubling time for NPCs is 96 hours[19]
10. The needed cell density for a single treatment is 2 X 10^7 cells[20]
11. There is a two day lag time for cells to adjust to their environment and attach to the growing area[21]
12. The differentiation time for iPSCs to NPCs is 12 days[22]
13. The mass of a cell is 1 ng[23]
14. The density of a cell is 1.05 g/mL

Reactors:

1. 80% daily replacement of media in each system in the bioreactor train to ensure proper nutrition and pH conditions for cells*
2. No losses from beginning vial to first well plate (WP-1)
3. Losses from WP-1 to second well plate (WP-2) are 5% to account for non-viability
4. Losses from WP-2 to the spinner reactor (BR-1) are 5% to account for non-viability
5. Losses from BR-1 to the second bioreactor (BR-2) are 15% to account for non-viability and incomplete transfer
6. Losses from BR-2 to the third bioreactor (BR-3) are 20% to account for non-viability, incomplete transfer, filtration and cleaving from the microcarriers
7. Losses from BR-3 phase 1 (neural induction) to BR-3 phase 2 (rosette selection) are 20% to account for non-viability, incomplete transfer, and filtration

[18] (Oh et al., 2009), Note: All "*" on this page are to denote that they came from this paper
[19] (Nunes et al., 2003)
[20] (EuroStemCell, 2015)
[21] (Bardy et al., 2013)
[22] (Technologies, 2011)
[23] (Mastin, 2009)

8. Losses from BR-3 phase 2 (rosette selection) and freezing are 5% for non-viability.

General:

1. Addition of 8 mg/mL of microcarriers for sufficient cellular growing area[24]
2. Microcarriers swell 15 mL/g[25]
3. Density and heat capacity of media is comparable to salt water (35 grams of salt / kilogram of water)[26]
4. The heat capacity of a cell is equivalent to the heat capacity of the human body which is 3.47 J/g-K[27]

Design Procedure

The following tables are a summary of our equipment. They are followed by descriptions, specifications, and design considerations behind each piece of equipment.

Table 6: Bio-reactor Equipment Summary

Equipment	Volume (mL)	Temperature (°C)	DO (% of air saturation)	pH	MoC	Growth Area (cm2)	Attachment Method	RPM
WP-1	2	37	30%	6.8-7.4	Plastic	8.8	Coated Matrix	N/A
WP-2	30	37	30%	6.8-7.4	Plastic	38.4	Coated Matrix	N/A
BR-1	350	37	30%	6.8-7.4	Glass	1080	Microcarriers	60
BR-2	500	37	30%	6.8-7.4	Glass	6480	Microcarriers	60
BR-3	5000	37	4%	6.8-7.4	Glass	N/A	Supsension	60

Table 7: Heating & Cooling Equipment Summary

Equipment	Peak Power (W)	Footprint (WxD)	Volume (L)	Temperature (°C)	MoC
F-1	1081	29.3"x24"	326	4	Cold-Rolled Steel
F-2	920	29.3"x24"	326	-20	Cold-Rolled Steel
F-3	2300	25.5"x30.75"	28	-50	Steel with Epoxy Finish
F-4	N/A	22" O.D.	71	-196	Powder Coated Steel
I-1	460	23.5"x18.5"	40	37	Cold-Rolled Steel
A-1	1400	21.5"x20"	19	121	Stainless Steel

Table 8: Auxiliary Equipment Summary

Equipment	Peak Power (W)	Footprint (WxD)	Volume (L)	MoC
UV-1	180	15.25"x20.5"	97	Stainless Steel
UV-2	105	25.75"x13.5"	316	Polypropylene
C-1	260	17.2"x22.4"	.72 or .042	Stainless Steel
WB-1	1032	15.5"x17"	10	Stainless Steel

[24] (Oh et al., 2009)
[25] (Sciences, 2011)
[26] (Laboratory, 2015)
[27] (Toolbox, 2016)

Table 9: Quality Control Equipment Summary

Equipment	Peak Power (W)	Footprint (WxD)
H-1	261	71"x31.5"
QC-1	25	15"x8.7"
QC-2	10	10.5"x25.5"

WP-1:

This system (WP-1) in the bio-reactor train is a single well plate that is made of polystyrene and is manufactured and shipped under sterile conditions. It is coated with an adherent matrix in order to provide adequate cell growth area. The two mL volume of the petri dish was determined based upon finding the volume required to seed the starting cell amount based on seeding density. It has a growth area of 8.8 cm^2. The petri dish is kept at 37 °C, under 30% air saturation for oxygen content, and a pH of 7.2 to ensure optimal growth conditions. The required daily media replacement for this system is 1.6 mL of growth media. This system is not agitated. This system is disposable.

WP-2:

This system (WP-2) in the bio-reactor train is a 6-well plate tray that is made of polystyrene and is manufactured and shipped under sterile conditions. It is coated with an adherent matrix in order to provide adequate cell growth area. The 30 mL volume of the 6-well plate tray was determined based upon finding the volume required to seed the starting cell amount based on seeding density. It has a growth area of 38.4 cm^2. The 6-well plate tray is kept at 37°C, under 30% air saturation for oxygen content, and a pH of 7.2 to ensure optimal growth conditions. The required daily media replacement for this system is 24 mL of growth media. This system is not agitated. This system is disposable.

BR-1:

This system (BR-1) in the bio-reactor train is a 100 mL spinner flask that is made out of glass and is manufactured and shipped under non-sterile conditions. Therefore, the spinner flask must be cleaned upon arrival. Cells grow on microcarriers added to the system which provide 1,080 cm^2 of growing area to the cells. The spinner flask is kept at 37 °C, under 30% air saturation for oxygen content, and a pH of 7.2 to ensure optimal growth conditions. The bio-reactor will be maintained under a 5% CO_2 atmosphere. This system is agitated by a magnetic stirrer. The rate of agitation will be manipulated to control the dissolved oxygen set point. The required daily media replacement for this system is 40 mL of growth media as the working volume of this reactor is 50 mL. This system is not disposable and it will require cleaning after each use before it may return to service.

BR-2:

This system (BR-2) in the bio-reactor train is a 500 mL reactor that is made out of glass and is manufactured and shipped under non-sterile conditions. Therefore, it will also require cleaning upon arrival to the facility. Cells grow on microcarriers added to the system which provide 6,480 cm^2 of growing area to the cells. The bio-reactor will be maintained under a 5% CO_2 atmosphere. The reactor is kept at 37 °C, under 30% air saturation for oxygen content, and a pH of 7.2 to ensure optimal growth conditions. This system is agitated by an impeller on the inside of the reactor tank. The rate of agitation will be manipulated to control the dissolved oxygen set point. The required daily media replacement for this system is 280 mL of growth media as the working volume of this reactor is only 350 mL. This system is not disposable and it will require cleaning after each use before it may return to service.

BR-3

This system (BR-3) in the bio-reactor train is a 5000 mL (5 L) reactor that is made out of glass and is manufactured and shipped under non-sterile conditions. Therefore, it like BR-2 and BR-1 will require cleaning upon arrival to the facility. No microcarriers are used in this reactor as the cells in this step form rosettes to induce differentiation. The bio-reactor will be maintained under a 5% CO_2 atmosphere. The reactor is kept at 37 °C, under 4% air saturation for oxygen content, and a pH of 7.2 to ensure optimal growth conditions. This system is agitated by an impeller on the inside of the reactor tank. The rate of agitation will be manipulated to control the dissolved oxygen set point. As explained above in the process description section this reactor is used twice. The first time it is used the daily media replacement for the system is 3,200 mL of neural induction media as it has a working volume of 4,000 mL (4 L). The second time it is used the daily media replacement for the system is 4,000 mL (4 L) of rosette selection media as it has a working volume of 5,000 mL (5 L). As this system is not disposable, it will require cleaning after each use before it may return to service. The cleaning of this system is critical and must be done efficiently as it will have the quickest turnaround time of any other step in this process as can be seen in Figure 2: High Production Gantt Chart

F-1:

This is a refrigerator that is capable of operating between 2 °C and 8 °C. It operates at 4 °C and contains parts of the growth media and the freezing preparation media. This refrigerator is sized so that it will be able to contain enough media to complete 12 batches should media shipments be disrupted. This will run four reactor trains for approximately three weeks.

F-2:

This is a freezer that is capable of operating between -20 °C and -30 °C and is held at -20 °C. It contains parts of the growth media and the neural induction media. This freezer is sized so that it will be able to contain enough media to complete 12 batches should media shipments be disrupted. This will run four reactor trains for approximately three weeks.

F-3:

This deep freezer is capable of operating between -50 °C and -80 °C and is held at -50 °C. Purified NPCs are placed within a temperature regulating cooling apparatus and subsequently placed within this freezer to cool from room temperature down to -50 °C.

F-4:

This is a liquid nitrogen freezer. It is used for the final cooling of purified NPCs and storage before shipment. It operates at -196 °C. This was designed to ensure that the packaged treatments did not have any biological activity, which stops at temperatures below -130 °C[28].

I-1:

The incubator is used to regulate the temperature of the well plates. It is set at 37 °C and operates year around. It has a 5% CO_2 atmosphere and 95% humidity to prevent the media from evaporating.

A-1:

This is the autoclave that is used to neutralize liquid wastes such as expended daily media. It is 19 L and is able to hold and entire batch's worth of media should contamination of that batch occur. It operates at 121 °C for 30 minutes to sterilize the waste.

UV-1:

This is the in-lab UV cabinet. It is used to sterilize neural induction media. It is also used to sterilize any potential solid biohazards before disposal.

UV-2:

This is the UV cabinet in the changing room that can be used to sterilize materials that cannot be soaked in ethanol but need to be brought into the clean room.

C-1:

This is the centrifuge. It is sized to fit at most four 180 mL centrifuge tubes and has a replaceable rotor in order to be used for smaller vial sizes such as when centrifuging vials during vial thaw. It is capable of holding 36 2 mL vials.

WB-1:

This is the water bath. It holds 10 L of water and is used to preheat media to 37 °C and thaw cells.

[28] (Kielberg, 2010)

H-1:

This is a laminar flow fume hood. It is used in several protocols in order to maintain aseptic technique when handling cells.

QC-1:

This is the cell counter used for quality control. It has the ability to count the total amount of cells in a sample as well as the non-viable amount of cells in a sample. These two numbers allow for the determination by difference of the total viable cell count per sample.

QC-2:

This is the cell analyzer used for quality control. It is a polymerase chain reaction (PCR) system that analyzes the RNA of the cells for specific gene expressions for positive identification of the cellular make-up when compared to a known standard.

Health & Safety

This facility was designed with consideration for the safety of the facility personnel and health of the future patients in each step of the design process. When looking at the hazards present in this facility they are significantly more nuanced than the majority of hazards that would be found in a refinery, chemical plant, or off-shore drilling rig. This makes a proper hazard analysis as well as appropriate consideration to contamination pathways paramount to the successful operation of the facility.

One could understand the temptation that exists to assume that since the hazards in this facility are unassuming and that the product is meant to be used in medicine that its production is benign. This however is most certainly not the case. The following section of this report will cover; proper manufacturing practices for the facility, identified hazards to the safety of plant personnel, potential contamination pathways that would jeopardize the safety of patients, the consequence of these occurrences, and steps that were taken to provide an inherently safe design with an appropriate amount of safeguards.

Current Good Manufacturing Practice (cGMP)

This facility was designed to meet the standards of current good manufacturing practice (cGMP) laid out by the United States Food and Drug Administration (FDA) which is charged with ensuring safe products and treatments are provided to the American Public. Notice that this facility is compliant with United States federal regulations and that upon determination of the location of the facility further investigation should be done to ensure state and local regulations are met. Different regulatory environments will exist outside of the United States and proper

consideration to these requirements should occur if the decision to open a facility outside of the United States is made.

The design criterion explicitly states that the facility needs to be cGMP compliant. However, when looking at the FDA's regulations the closest category that this facility fit would be a "Phase 1 Investigational Drug". Looking closer at the industry guidance for this category it is revealed that "this guidance does ***not*** apply to the following phase 1 investigational products: Human cell or tissue products regulated solely under § 361 of the Public Health Service Act"[29]. Occasionally there are requirements that cGMP does not sufficiently cover in the required procedures to meet federal regulation. Therefore, pursuant to § 361 of the Public Health Service Act this facility must follow current good tissue practice (cGTP) to ensure that it is always in compliance with federal regulation[30]. In more simple terms, because this facility produces human cells for use in cell therapies it must follow the more stringent requirement of cGTP which will also satisfy good cGMP requirements.

After reading the Industry Guidance for "Current Good Tissue Practice (CGTP) and Additional Requirements for Manufactures of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)" compiled by the FDA the following design considerations were made to ensure proper manufacturing of stem cells from this facility. This design meets the core cGTP requirements as outlined below.

All of the manufacturing steps occur in a 25 ft. by 36 ft. (900 ft^2) ISO Class 3 clean room. The clean room is outfitted such that it has controls for temperature and humidity, adequate ventilation and air filtration, and is able to maintain conditions that allow for equipment to operate in an aseptic process. The facility is cleaned using 70% ethanol for intentional decontamination of new materials entering the clean room. Employees are provided with an antechamber to change into lab clothes before entering the clean room proper.

While the majority of the manufacturing process occurs in the main section of the clean room, mix-ups of cells will be prevented with procedures and the volume differences inherent at the different steps. Furthermore, the storage location for cells ready to be picked up is a separate section from the rest of the clean room.

All purchased equipment will meet qualification requirements and be of a design that limits the chances of contaminating any of the samples. All protocols will follow the manufacturer's procedures for proper usage. Equipment will be properly maintained and regularly inspected and calibrated.

All supplies and reagents, unless otherwise stated, used in this process will be purchased from manufactures that provide sterile non-human based products for the facility. A major exception

[29] (United States, 2008)
[30] (United States, 2011)

to this is the neural induction media which will come from human-feeders and thus will be irradiated in UV-1 in order to inactive any potential virus before being used in the process[31].

No recovery is expected to occur within the facility as the cells required to start the process are shipped in a sterile condition to the facility. This design is not prepared to handle recovery operations and as a result no recovery operations should occur within the facility.

It is not expected that this facility will encounter dura mater or transmissible spongiform encephalopathy (TSE). While the facility does deal with developing cells for treatment in spinal injuries, the cells provided from donors that initiate each process are iPSCs that could have been acquired from almost anywhere in the donor's body. It is assumed that the cells were not acquired from the donor's spinal cord or brain. Lastly, NPCs are not known to differentiate into dura mater.

Testing of the cells manufactured in this facility occurs at the end of the expansion phase, which occurs after BR-2, and at the end of the differentiation phase, which occurs after the second instance of the cells in BR-3. The quality control tests and protocol can be further read about in the section of this report called "Other Important Considerations."

Labeling practices of the facility will follow the industry standard and insure that the labels are accurate, legible and maintain the integrity of the label.

Storage facilities on-site will be independently temperature controlled from the clean room and proper labeling will ensure that expired material is discarded so as to not enter the manufacturing process.

Disposal of wastes from this facility occurs in one of two ways dependent upon if the waste is a liquid or solid. Liquid wastes, such as the daily expended media, are placed within a 19 L Autoclave kill tank (A-1) and heated to 121°C for 30 minutes in order to ensure all biological wastes are destroyed. The pH of the solution is checked with litmus paper to ensure that the solution has a pH between 5 and 9. The solution is anticipated to be near neutral but hydrochloric acid and sodium hydroxide are on-site for any necessary neutralization. After autoclaving and neutralization the solution is poured down the sink to the sewer system. Solid wastes are soaked in 70% ethanol for 30 minutes before being discarded. Alternatively, some solid wastes may be cleaned utilizing the UV cabinet on-site.

Hazard Analysis

Material Properties

[31] (Yen et al., 2014)

Of the chemicals found within the facility the majority are of proprietary blend and therefore only list material properties if they are dangerous. None of the media had dangers listed. Outside the media, Table 10: Material Properties contains material properties of chemicals of interest.

Table 10: Material Properties

Material Properties	MW (g/mol)	Boiling Point (°C)	Liquid Density (kg/L)	Reactivity With Water	Toxicity Limits
Water	18.01	100.00	1	n/a	>90 (ml/kg)
Carbon Dioxide	44.01	-78.50	n/a	n/a	non-toxic
Liquid Nitrogen	28.01	-196.00	0.807	n/a	non-toxic
Sodium Bicarbonate	84.01	n/a	2.159	n/a	>3360 (mg/kg)
Hydrochloric Acid (20%)	36.46	108.58	1.2	yes	900 (mg/kg)
Sodium Hydroxide	40.00	1388.00	2.13	yes	>500 (mg/kg)

Interaction Matrix

The provided interaction matrix seen below contains the majority of the chemical components that are anticipated to be in the facility. Exact make-up of the different media is unknown, but Phosphate Buffer Saline was used as a close approximation of what can be expected. There appears to be several concerning reactions, however it was determined that the program is assuming that sizeable quantities of solid material would be available to react. This is not the case for this facility as these chemicals are dissolved and not expected to have significant interactions with their surroundings. This interaction matrix was developed using the NOAA software and CRW3 program.

Mixture Manager
Compatibility Chart

NFPA Health	NFPA Flammability	NFPA Instability	NFPA Special	PBS Compatibility Chart	AIR, COMPRESSED	CARBON DIOXIDE	DIBASIC SODIUM PHOSPHATE	HYDROCHLORIC ACID, SOLUTION	NITROGEN, REFRIGERATED LIQUID (CRYOGENIC LIQUID)	POTASSIUM CHLORIDE	SODIUM BICARBONATE	SODIUM CHLORIDE	WATER
				AIR, COMPRESSED									
				CARBON DIOXIDE	Y								
				DIBASIC SODIUM PHOSPHATE	N	Y							
3	0	1		HYDROCHLORIC ACID, SOLUTION	N	Y	N						
3	0	0		NITROGEN, REFRIGERATED LIQUID (CRYOGENIC	Y	Y	Y	Y					
				POTASSIUM CHLORIDE	N	Y	C	N	Y				
				SODIUM BICARBONATE	N	Y	Y	N	Y	Y			
				SODIUM CHLORIDE	N	Y	C	N	Y	Y	Y		
				WATER	N	Y	C	C	Y	Y	C	Y	

Figure 5: Interaction Matrix

Safety Assessment Results

Hazard Identification & Potential Consequence Summary

Table 11: Hazard & Potential Consequence Summary

Hazard No.	Hazard		Equipment Damage	Process Disruption	Economic Impact	Legal/PR
1	Virus in Treatment		-	-	high	high
2	Contamination Pathway:	Non-Aseptic Technique	-	high	medium/low	-
3		Clean Room Breach	low	high	high	-
4		Tainted Media	-	high	medium/low	-
5	Unsecured Gas Cylinder		high	high	high	-
6	Liquid Nitrogen Spill		low	medium	high/medium	-
7	HCl/NaOH Spill		low	low	-	-
8	Technician Inattention		high	high	high/medium	-
9	QC Failure		-	-	high	high
10	Improper Differentiation		-	high	medium/low	-
11	Incorrect Media		-	high/medium	high/medium	-
12	Improper Biohazard Disposal		-	-	low	medium

Table 11: Hazard & Potential Consequence Summary contains several hazards that were identified in the process along with the potential consequences of those hazards.

Hazard 1 is if a virus were to make it through the facility and into a treatment that was then used during surgery and led to a patient suffering harm in some fashion. This would have little impact on the equipment or process but would be disastrous economically and legally.

Hazards 2, 3, and 4 are all contamination pathways that would lead to the contaminated batch being destroyed when it was determined to be contaminated by quality control.

Hazard 5 assumes that a gas cylinder under pressure is not secured and begins to discharge. This could cause serious damage to the equipment and disrupt the process potentially leading to large economic losses.

Hazards 6 and 7 could result in potential equipment damage.

Hazard 8 occurs when a technician becomes complacent in the workplace and potential damage to equipment or contamination could occur should the technician stop following the proper protocols.

Hazard 9 would be just as impactful as Hazard 1 and would lead to great economic loss and legal trouble.

Hazards 10, 11 and 12 are all potential hazards that would ruin a batch of cells and are therefore undesirable but do not appear to place anyone in harm's way.

Safeguards

Table 12: Inherently Safer Design Application Summary

Hazard No.	Hazard		Inherient Safety/User Friendly Concept	Incorperation
1	Virus in Treatment		Procedure	QC tests
			Limitation of Effects	Batch Disposal
			Avoiding Domino Effect	Multiple Reaction Trains
4	Contamination Pathway	Tainted Media	Procedure	UV Sterilization
6	Liquid Nitrogen Spill		Minimization	Limited Liqid Nitrogen Usage
			Limitation of Effects	Separated Liquid Nitrogen Area
7	HCl/NaOH Spill		Minimization	Limited Need for HCl/NaOH
10	Improper Differentiation		Procedure	Filtering Rosettes
12	Improper Biohazard Disposal		Procedure	UV and Autoclave Sterilization

Table 12: Inherently Safer Design Application Summary shows which hazards have been systematically mitigated by the design of this facility. It focuses on minimizing hazardous material being used and proper procedures being followed.

Table 13: Opportunities for Additional Design Safeguards

Hazard No.	Hazard		Considerations
2	Contamination Pathway:	Non-Aseptic Technique	Aseptic Training and Procedure
3		Clean Room Breach	Clean Room Training and Disinfection Protocol
5	Unsecured Gas Cylinder		Secure Gas Cylinders
8	Technician Inattention		Training and Monitoring
9	QC Failure		QC Training and Procedures
11	Incorrect Media		Proper Labeling and Storage System

Table 13: Opportunities for Additional Design Safeguards addresses the hazards that need to be evaluated in detailed design. The majority of these safety recommendations center on proper training of the technicians as well as protocol being followed.

Inherent Safety Design Summary

The result of the hazard analysis indicates that there are some potentially catastrophic events such as virus infection of a patient or quality control failure. These hazards have been reduced through an inherently safe design but can further mitigated through procedures and training established in detailed design. None of these hazards present potential for project termination. The only major concern that requires special attention is maintaining the sterility of the clean room and manufacturing process. Overall, this process does not pose immediate risk to those involved in the day-to-day operation of the facility and is a safe operation.

Economic Analysis

The standalone assumption has been made for economic analysis of the project. This is because the company is a small to midsized company with an absence of other projects that may have produced other taxable revenue that could be reduced by losses incurred from this project.

Beyond the operational difficulties of handling large volumes and maintaining thermal and nutrient ubiquity throughout large reactors with low mixing speeds there exists economic rationale for limiting reactor size. The economic rationale is to limit volume levels in order to mitigate the economic losses and business disruption that occurs should a batch become contaminated. This is achieved by limiting reactor volume and meeting production requirements with reduced downtime between batches and multiple reactor trains.

The module system and the ability to mix and match reactor train totals and production rates allows for cell production levels to respond to market forces. The initial capital investment will allow for the creation of the first module with a single reactor train. While the price per treatment of this set-up will be higher than if all four reactor trains were being used, it allows for initial treatments and production to occur in order to prove the viability of this cell therapy to the broader market. As demand for cell production increases more reactor trains can be added to the module. Since the internal rate of return (IRR) is fixed at 50% the more treatments produced per module the cheaper the treatment can be sold.

If any of the following events or combination of events occurs:

a) The original module is unable to meet market demand

b) A sufficient market demand develops in a location distant from the original module

c) Regulatory constraints on production, transportation or sale of the treatment in new markets

Then new modules can be produced in those areas to address the events as they occur. The above list of events is simply speculation on the most likely explanations for the addition of another module and should not be considered exhaustive.

There are many ways to operate this facility and the economic analysis covers three different scenarios. The first scenario is a single module operating with one reactor train at low production. The second scenario is a single module operating with one reactor train at high production. The third and final scenario is a single module operating with four reactor trains at high production. Any combination between scenario one and three should be acceptable. An economic analysis was not provided for all possible scenarios as the expected economic outcome will fall between the economics of the first and third scenarios.

Table 14: Economic Analysis Summary

	Production Type	Annual Treaments	Treatment Price	Percent Price Reduction
Scenario 1	Low Production, 1 Train	1,485	$ 2,466	-
Scenario 2	High Production, 1 Train	4,471	$ 1,005	59%
Scenario 3	High Production, 4 Train	17,882	$ 508	79%

Table 14: Economic Analysis Summary provides a simple direct comparison of the different scenario production outcomes. It proves the annual treatments produced the cost that each treatment would need to be sold in order to achieve a 50% IRR and how much cheaper the treatment gets with increased production.

Capital Cost Estimates

Due to the unique scope of the project, the capital cost estimates are based on current prices for equipment that is similar to what is needed to operate the manufacturing facility. The selected equipment meets all the specifications needed for the process, but is as small as is feasible in order to minimize the size of the clean room. The clean room and all non-disposable equipment that the cells or the media need in order to be processed is included in the capital cost estimates. All prices obtained are current prices, and since equipment for the manufacturing facility will be purchased this year in order to prepare for operation next year, the prices were not escalated.

Since the equipment is not large or complex, and all equipment used for reference had set up and operation guides, it was assumed that the technicians hired are able to properly set up the equipment. Therefore, no cost was added for installation cost. However, to cover any fees associated with purchasing or shipping a cost of 3% was added to the base purchase price of the equipment. To cover any contingencies in purchasing or delivering the equipment 15% was added to the base purchase price.

Capital costs are split into two categories: module equipment and reactor train equipment. Module equipment is any equipment that is only purchased for a new module and will be used regardless of how many reactor trains are operating. Reactor train equipment consists of all equipment that must be purchased for each reactor train that is operating in the module as each reaction train needs a unique set of equipment to operate efficiently.

Table 15: Fixed Capital Investment per Module

Item	Price	Price with Contengencies and Fees
Clean Room	$ 729,000	$ 860,220
UV Sterilizer (UV-1)	$ 1,152	$ 1,359
UV Cabinet (UV-2)	$ 4,602	$ 5,430
Liquid Nitrogen Freezer (F-	$ 6,095	$ 7,192
Deep Freezer (F-3)	$ 10,406	$ 12,279
Freezer (F-2)	$ 7,292	$ 8,605
Refrigerator (F-1)	$ 5,559	$ 6,559
Laminar Flow Hood (H-1)	$ 8,349	$ 9,852
Autoclave (A-1)	$ 5,760	$ 6,797
Large Centerfuge (C-1)	$ 1,395	$ 1,646
Water Bath (WB-1)	$ 467	$ 551
Incubater (I-1)	$ 5,322	$ 6,280
Cell Counter (QC-1)	$ 15,000	$ 17,700
Cell Analyzer (QC-2)	$ 30,900	$ 36,462
	Total FCI/Module	$ 980,932

Table 16: Fixed Capital Investment per Reactor Train

Item	Price	Amount Needed	Total	Total with Contengencies and Fees
Bio-reactor 1 w/ pH	$ 1,152.44	1	$ 1,152.44	$ 1,359.88
Bio-reactor 2 (BR-2)	$ 21,942.68	1	$ 21,942.68	$ 25,892.36
Bio-reactor 3 (BR-3)	$ 52,094.24	1	$ 52,094.24	$ 61,471.20
Cooling Containers	$ 106.00	9	$ 954.00	$ 1,125.72
			Total FCI/Train	$ 89,849.16
			Total FCI for 1 Train	$ 1,070,781.37
			Total FCI for 4 Trains	$ 1,340,328.87

Table 15: Fixed Capital Investment per Module above lists the estimated price for the capital costs along with the total cost after contingencies and fees for the module equipment. While Table 16: Fixed Capital Investment per Reactor Train above lists the estimated price for the capital costs for the reactor train equipment. The final investment for both the single train and four train systems are totaled at the bottom of the Table 16: Fixed Capital Investment per Reactor Train and are referred to as the fixed capital investment (FCI) in the rest of the report.

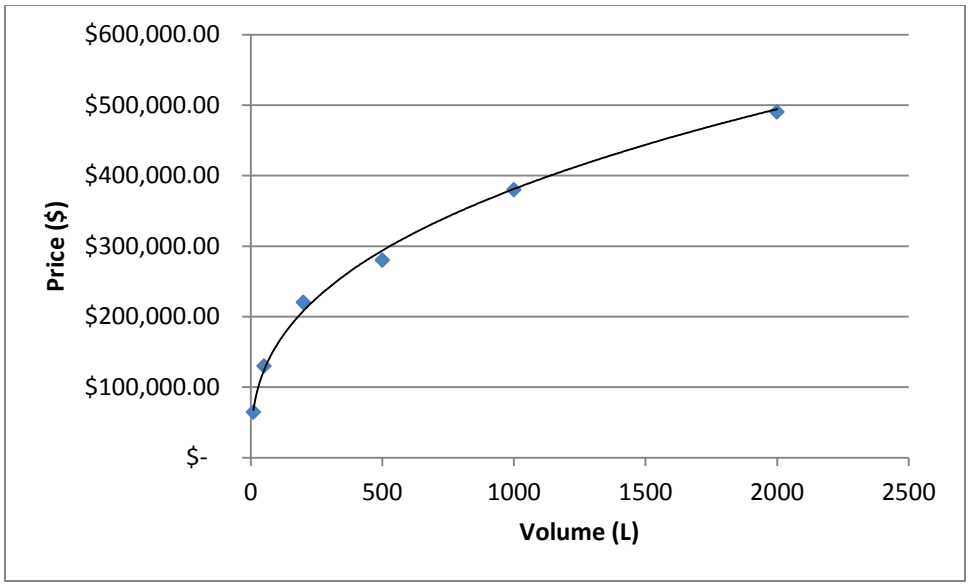

Figure 6: Bio-reactor Price Correlation

The bio-reactor price was determined from the provided economics spreadsheet from GE by modeling the prices as a function of volume. A correlation was established, as seen in Figure 6: Bio-reactor Price Correlation, and used to estimate the cost of a 5 L and a 0.5 L reactor as they were not offered as sizes on the spreadsheet. The calculated prices are shown in Table 16: Fixed Capital Investment per Reactor Train.

Manufacturing Cost Estimates

The manufacturing costs for this facility were determined on a per batch basis and a per module basis. This was done because increasing production rates only reduces downtime it does not change the total cost to produce any individual batch. More simply put, operating at low production rates provides 10 batches per year and at high production rates is 30 batches per year, regardless of how many batches are produced that year the manufacturing costs to produce that batch are unchanged. There are still some costs that are incurred for simply operating a module regardless of how many batches are produced from that module. In order to calculate the price per year for batch costs one must simply multiple the total batch cost by the amount of batches produced in that year.

Table 17: Raw Material Costs per Module

Item	Price/batch
Adult Stem Cells	$587
Growth Media	$1,338
Neural Induction Media	$19,118
Rosette Selection Media	$10,150
Cryopreservation Media	$607
Total	$31,799

The raw material costs per batch can be seen in Table 17: Raw Material Costs per Module. This includes the initial vial of cells and all media required for the expansion, differentiation, and storage of the cells.

Table 18: Operating Cost per Batch

Item	Price/batch
TrypLE	$ 58.22
Microcarriers	$ 11.94
Microcarrier Prep	$ 52.92
Well Plate 1	$ 0.80
Well Plate 2	$ 11.94
40 Micron Cell Strainer	$ 33.28
100 Micron Cell Strainer	$ 33.28
400 Micron Cell Strainer	$ 33.28
50 mL Centerfuge Tubes	$ 11.36
Cryovials	$ 105.85
Cryocontainers	$ 0.31
NucleoCassettes	$ 14.80
Reagent A100	$ 0.07
Reagent B100	$ 0.07
QC Growth Wellplate	$ 568.00
QC Differentiation Wellplate	$ 568.00
RNA Kit	$ 46.80
Ethanol	$ 54.96
Total	$ 1,605.89

The operating costs per batch can be seen in Table 18: Operating Cost per Batch**.** This includes the disposable materials such as the well plates, microcarriers, chemicals and intermediate cell holders. The prices have been left unrounded to the penny, as rounding of this table would cause significant distortion to the table.

Table 19: Operating Cost per Module

Item	Cost/Year
CO2	$ 30
Liquid Nitrogen	$ 369
Disposables	$ 20,000
Total	$ 20,399

The operating costs per module can be seen in Table 19: Operating Cost per Module**.** These costs are incurred when a single batch is run and are mostly independent of the production rate of the facility. These costs contain an estimation of the total disposables used to operate a laboratory and should be sufficient to cover all of the production rates[32].

Table 20: Utility Cost Per Batch

Electricity Usage	**Power (kW)**	**Hours/Batch**	**Cost/Batch**
Bio-reactor 1 (BR-1)	1.05	180.00	$ 9.45
Bio-reactor 2 (BR-2)	0.418	179.28	$ 3.75
Bio-reactor 3 (BR-3)	0.418	288.00	$ 6.02
Autoclave (A-1)	1.4	1.50	$ 0.11
Centerfuge (C-1)	0.26	0.03	$ 0.00043
Cell Counter (QC-1)	0.01	2.40	$ 0.0012
Cell Analyzer (QC-2)	0.025	0.03	$ 0.00004
Water Bath (WB-1)	1.032	2.13	$ 2.19
Water	**Volume (L)**		
Rinsing Water	9.85		$ 9.85
Autoclave (A-1 Water Consumption)	1.65		$ 1.65
		Total	$ 33.02

The utility costs per batch can be seen in Table 20: Utility Cost Per Batch**.** This includes the electricity to operate all equipment and water consumption.

[32] (Veronese, 2011)

Table 21: Utility Costs per Module

Electricity Usage	**Power (kW)**	**Hours/Batch**	**Cost/year**	
Fume Hood	0.261	8760	$	114.32
Incubater	0.46	8760	$	201.48
Refrigerator	1.081	2920	$	157.83
Freezer	0.92	2920	$	134.32
Deep Freezer	2.3	2920	$	335.80
UV Irradiator	0.18	1073.1	$	9.66
UV Cabinet	0.105	8760	$	45.99
Water Bath Heating Water	1.032	141.255	$	7.29
Cell Counter Standby	0.000002	8760	$	0.00088
Water	**Volume (L)**		**Cost/year**	
Water Bath	3650		$	1.98
Water for Injection	**Volume (L)**		**Cost/year**	
Incubater	295.23		$	295.23
		Total	$	1,303.89

The utility costs per module can be seen in Table 21: Utility Costs per Module. This shows the utility costs that are incurred by simply operating the module and these costs are independent of production rates.

Table 22: Waste Treatment Costs

Waste Treatment Cost per Batch				
Sewage	**Volume (L)**		**Cost/Batch**	
Rinsing Water	9.85		$	0.01
Media After Autoclave	55.4368		$	0.07
Water for Autoclave	1.65		$	0.002
		Total	$	0.09
Waste Treatment Cost per Module				
Sewage	**Volume (L)**		**Cost/year**	
Water Bath	3650		$	4.83
		Total	$	4.83

The waste treatment cost per batch and per module is provided in Table 22: Waste Treatment Costs.

Table 23: Labor Cost Summary

	Required Workers/Shift	Number of People Hired	Salary/Year	Total cost/Year
Technicians	2	9	$41,290.00	$ 371,610.00
Scientists	1	5	$79,930.00	$ 399,650.00
				$ 771,260.00
Additional Technician	1	5	$41,290.00	$ 206,450.00
				$ 977,710.00

The labor costs were evaluated via data provided by the Bureau of Labor Statistics[33,34]. It was determined that each shift would require at least 2 technicians and 1 medical scientist. An additional technician is added when the number of trains in the module is three or four. The number of people hired to cover each shift is taken from Turton and assumes that the facility operates 24 hours a day[35].

Table 24: Total Manufacturing Costs without Depreciation

Direct Manufacturing Costs	Scenario 1	Scenario 2	Scenario 3	
Production Type	Low Production, 1 Train	High Production, 1 Train	High Production, 4 Train	Estimation Factor
Raw Material Cost/Year, C_{RM}	$ 319,131	$ 960,561	$ 3,842,246	n/a
Waste Treatment Cost/Year, C_{WT}	$ 6	$ 8	$ 16	n/a
Utilities Cost/Year, C_{UT}	$ 1,635	$ 2,301	$ 5,293	n/a
Operating Labor, C_{OL}	$ 771,260	$ 771,260	$ 977,710	n/a
Direct Supervisory and Clerical Labor	$ 138,827	$ 138,827	$ 175,988	$0.18C_{OL}$
Maintainance and Repairs	$ 64,247	$ 64,247	$ 80,420	0.06FCI
Operating Supplies	$ 36,515	$ 68,908	$ 214,435	n/a
Laboratory Charges	$ 77,126	$ 77,126	$ 97,771	Low Estimate $0.1C_{OL}$
Patents and Royalties	$ 80,510	$ 105,491	$ 235,643	$.03*COM_d$
Total Direct Manufacturing Costs	$ 1,408,747	$ 2,083,238	$ 5,393,878	
Fixed Manufaturing Costs				
Local Taxes and Insurance	$ 34,265	$ 34,265	$ 42,891	0.032FCI
Lab Overhead Costs	$ 584,600	$ 584,600	$ 740,471	$0.708C_{OL}$+0.036FCI
Total Fixed Manufacturing Costs	$ 618,865	$ 618,865	$ 783,361	
General Manufacturing Expenses				
Administration Costs	$ 146,150	$ 146,150	$ 185,118	$0.177C_{OL}$+0.009FCI
Distribution and Selling Costs	$ 295,202	$ 386,800	$ 864,024	$.11*COM_d$
Research and Development	$ 134,183	$ 175,818	$ 392,738	$.05*COM_d$
Total General Manufacturing Costs	$ 146,150	$ 146,150	$ 185,118	
Total Costs	$ 2,173,762	$ 2,848,253	$ 6,362,357	
COM_d	$ 2,683,657	$ 3,516,362	$ 7,854,761	Total Costs/.81

Using the method described by Turton, the total cost of manufacturing was calculated for the three different scenarios[36]. This can be seen Table 24: Total Manufacturing Costs without Depreciation.

Table 25: Batch Summary per Year

	Production Type	Annual Treaments	Batches/Year
Scenario 1	Low Production, 1 Train	1,485	10.04
Scenario 2	High Production, 1 Train	4,471	30.21
Scenario 3	High Production, 4 Train	17,882	120.83

[33] (Statistics, 2014a)
[34] (Statistics, 2014b)
[35] (Turton, Bailie, Whiting, & Shaeiwitz, 2009)
[36] (Turton et al., 2009)

Lastly, Table 25: Batch Summary per Year contains a summary of the different proposed operational production rates. It compares Scenarios 1, 2 and 3.

Treatment Price

Since the IRR is fixed, the treatment price varies with the total cell production. As production is increased and the fixed capital cost remains unchanged more cells are produced at a disproportionately cheaper price. This causes the price per treatment to decrease drastically. This can be seen in the cash flow sheets for Scenario 1 in Table 26: Cash Flow Sheet for Scenario 1, Scenario 2 in Table 27: Cash Flow Sheet for Scenario 2**,** and Scenario 3 in Table 28: Cash Flow Sheet for Scenario 3.

Table 26: Cash Flow Sheet for Scenario 1

Project Title:	Low Production of Stem Cell Treatments							
Corporate financial situation:	Stand Alone							
Minimum rate of return, i* =	0.1	or	10.0%					
Other relevant project info.	5 Year MACRS Depreciation							
1 = $1								
End of Year	2016	2017	2018	2019	2020	2021	2022	2023
Number of treatments		743	1,485	1,485	1,485	1,485	1,485	1,485
x Sales Price, $/treatment		2,466	2,466	2,466	2,466	2,466	2,466	2,466
Net Revenue	-	1,831,188	3,662,377	3,662,377	3,662,377	3,662,377	3,662,377	3,662,377
- Cost of Manufacturing		(1,341,828)	(2,683,657)	(2,683,657)	(2,683,657)	(2,683,657)	(2,683,657)	(2,683,657)
- Depreciation		(214,156)	(342,650)	(205,590)	(123,354)	(123,354)	(61,677)	-
- Loss Forward			-	-	-	-	-	-
Taxable Income	-	275,204	636,070	773,130	855,366	855,366	917,043	978,720
- Tax @ 40%	-	(110,082)	(254,428)	(309,252)	(342,146)	(342,146)	(366,817)	(391,488)
Net Income	-	165,122	381,642	463,878	513,220	513,220	550,226	587,232
+ Depreciation	-	214,156	342,650	205,590	123,354	123,354	61,677	-
+ Loss Forward	-	-	-	-	-	-	-	-
- Fixed Capital	(1,070,781)	-	-	-	-	-	-	-
Cash Flow	(1,070,781)	379,279	724,292	669,468	636,574	636,574	611,903	587,232
Discount Factor ($P/F_{i^*,n}$)	1.0000	0.9091	0.8264	0.7513	0.6830	0.6209	0.5645	0.5132
Discounted Cash Flow	(1,070,781)	344,799	598,588	502,981	434,788	395,262	345,403	301,343
NPV @ i* =	1,852,384							
DCFROR =	50%							
Paybock Period =	2.25	years						

Table 27: Cash Flow Sheet for Scenario 2

Project Title:	One Train High Production of Stem Cell Treatments							
Corporate financial situation:	Stand Alone							
Minimum rate of return, i* =	0.1	or	10.0%					
Other relevant project info.	5 Year MACRS Depreciation							
1 = $1								
End of Year	2016	2017	2018	2019	2020	2021	2022	2023
Number of treatments		2,235	4,471	4,471	4,471	4,471	4,471	4,471
x Sales Price, $/treatment		1,005	1,005	1,005	1,005	1,005	1,005	1,005
Net Revenue	-	2,247,541	4,495,082	4,495,082	4,495,082	4,495,082	4,495,082	4,495,082
- Cost of Manufacturing		(1,758,181)	(3,516,362)	(3,516,362)	(3,516,362)	(3,516,362)	(3,516,362)	(3,516,362)
- Depreciation		(214,156)	(342,650)	(205,590)	(123,354)	(123,354)	(61,677)	-
- Loss Forward			-	-	-	-	-	-
Taxable Income	-	275,204	636,070	773,130	855,366	855,366	917,043	978,720
- Tax @ 40%	-	(110,082)	(254,428)	(309,252)	(342,146)	(342,146)	(366,817)	(391,488)
Net Income	-	165,122	381,642	463,878	513,220	513,220	550,226	587,232
+ Depreciation	-	214,156	342,650	205,590	123,354	123,354	61,677	-
+ Loss Forward	-	-	-	-	-	-	-	-
- Fixed Capital	(1,070,781)	-	-	-	-	-	-	-
Cash Flow	(1,070,781)	379,279	724,292	669,468	636,574	636,574	611,903	587,232
Discount Factor ($P/F_{i^*,n}$)	1.0000	0.9091	0.8264	0.7513	0.6830	0.6209	0.5645	0.5132
Discounted Cash Flow	(1,070,781)	344,799	598,588	502,981	434,788	395,262	345,403	301,343
NPV @ i* =	1,852,384							
DCFROR =	50%							
Paybock Period =	2.25	years						

Table 28: Cash Flow Sheet for Scenario 3

Project Title:	Four Train High Production of Stem Cell Treatments							
Corporate financial situation:	Stand Alone							
Minimum rate of return, i* =	0.1	or	10.0%					
Other relevant project info.	5 Year MACRS Depreciation							
1 = $1								
End of Year	2016	2017	2018	2019	2020	2021	2022	2023
Number of treatments		8,941	17,882	17,882	17,882	17,882	17,882	17,882
x Sales Price, $/treatment		508	508	508	508	508	508	508
Net Revenue	-	4,539,927	9,079,854	9,079,854	9,079,854	9,079,854	9,079,854	9,079,854
- Cost of Manufacturing		(3,927,381)	(7,854,761)	(7,854,761)	(7,854,761)	(7,854,761)	(7,854,761)	(7,854,761)
- Depreciation		(268,066)	(428,905)	(257,343)	(154,406)	(154,406)	(77,203)	-
- Loss Forward			-	-	-	-	-	-
Taxable Income	-	344,481	796,188	967,750	1,070,687	1,070,687	1,147,890	1,225,093
- Tax @ 40%	-	(137,792)	(318,475)	(387,100)	(428,275)	(428,275)	(459,156)	(490,037)
Net Income	-	206,688	477,713	580,650	642,412	642,412	688,734	735,056
+ Depreciation	-	268,066	428,905	257,343	154,406	154,406	77,203	-
+ Loss Forward	-	-	-	-	-	-	-	-
- Fixed Capital	(1,340,329)	-	-	-	-	-	-	-
Cash Flow	(1,340,329)	474,754	906,618	837,993	796,818	796,818	765,937	735,056
Discount Factor ($P/F_{i^*,n}$)	1.0000	0.9091	0.8264	0.7513	0.6830	0.6209	0.5645	0.5132
Discounted Cash Flow	(1,340,329)	431,595	749,271	629,597	544,237	494,761	432,351	377,200
NPV @ i* =	2,318,683							
DCFROR =	50%							
Paybock Period =	2.25	years						

The project evaluation life was assumed to be 7 years as this gives a reasonable range to produce revenue that offsets the initial capital investment. A 5 year MACRS deprecation was used as this facility is made up primarily of laboratory and research equipment. In accordance with MACRS, the assumption is made that the facility is only in production for half a year for the

first year. The Net Present Value (NPV) for Scenarios 1 & 2 is 1.85 million dollars. The NPV for Scenario 3 is 2.32 million dollars. All scenarios meet the design criteria of a 50% IRR.

A direct comparison of the critical economic considerations can be seen in Table 14: Economic Analysis Summary. Notice that producing cells in Scenario 1 provides the most expensive treatment cost. Scenario 3 offers a treatment cost that is nearly 80% cheaper than in Scenario 1. However, Scenario 3 produces 12 times the amount of treatments. This should be considered as this cell therapy treatment makes its way onto the market place. Allowing market forces to dictate cell production will be the best protection against over production. This means that initially the treatments will remain relatively expensive to what they could cost as the first few thousand patients undergo treatment. As the market demand for treatments increases cellular production should be increased.

Average Yearly Expenses

Severity of Injury	First Year	Each Subsequent Year
High Tetraplegia (C1-C4) ASIS ABC	$1,064,716	$184,891
Low Tetraplegia (C5-C8)	$769,351	$113,423
Paraplegia	$518,904	$68,739
Incomplete motor function at any level	$347,484	$42,206

Lengths of stay in the hospital acute care unit have declined from 24 days in the 1970s to 11 days currently. Rehabilitation lengths of stay have also declined from 98 days in the 1970s to 36 days currently.

Estimated Lifetime Costs by Age of Injury

Severity of Injury	25 Years Old	50 Years Old
High Tetraplegia (C1-C4) ASIS ABC	$4,724,181	$2,596,329
Low Tetraplegia (C5-C8) ASIS ABC	$3,451,781	$2,123,154
Paraplegia ASIS ABC	$2,310,104	$1,516,052
Incomplete motor function at any level ASIS D	$1,578,274	$1,113,990

Data Source: Economic Impact of SCI published in the journal Topics in Spinal Cord Injury Rehabilitation, Volume 16, Number 4, in 2011.
ASIA Impairment Scale (AIS) is used to grade the severity of a person's neurological impairment following spinal cord injury.

Figure 7: Estimated Spinal Cord Injury Treatment Costs

The approximated annual and lifetime cost for individuals disabled due to spinal cord injury of varying severity is provided in the above Figure 7: Estimated Spinal Cord Injury Treatment Costs[37]. The above graphic was provided by the Christopher & Dana Reeve Foundation.

As can be seen when comparing the price per treatment to the expected costs for just the annual costs of caring for an individual suffering from a spinal cord injury let alone the lifetime costs it is clear that the cell therapy facility designed in this report will offer a superior

[37] (Foundation, 2015)

alternative to healthcare professionals and their patients in dealing with spinal cord injuries. The cost to produce the cells required for treatment is orders of magnitudes cheaper.

Sensitivity Analysis

A sensitivity analysis was performed in order to determine the effect of cost variability on the price of the treatment.

Tornado Charts

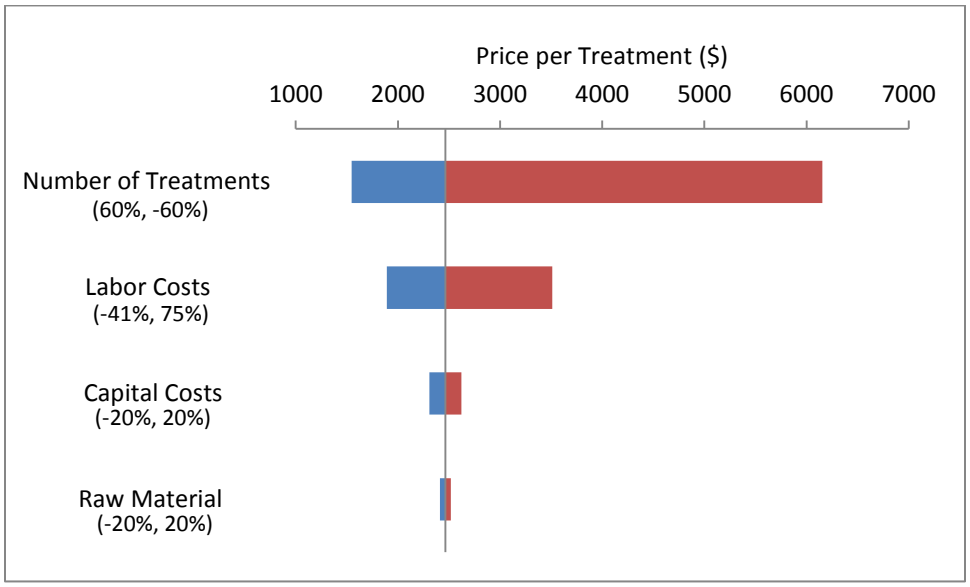

Figure 8: Scenario 1 Tornado Chart

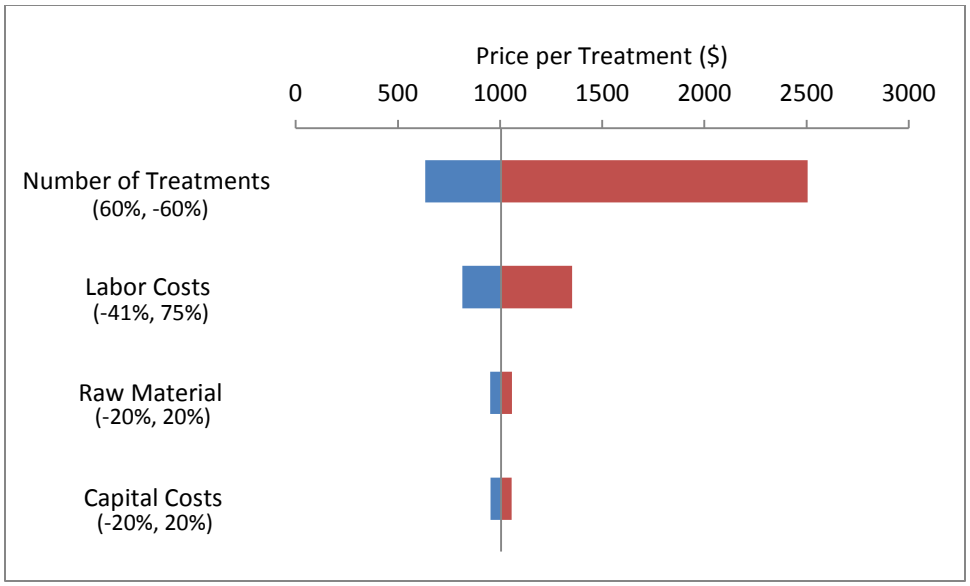

Figure 9: Scenario 2 Tornado Chart

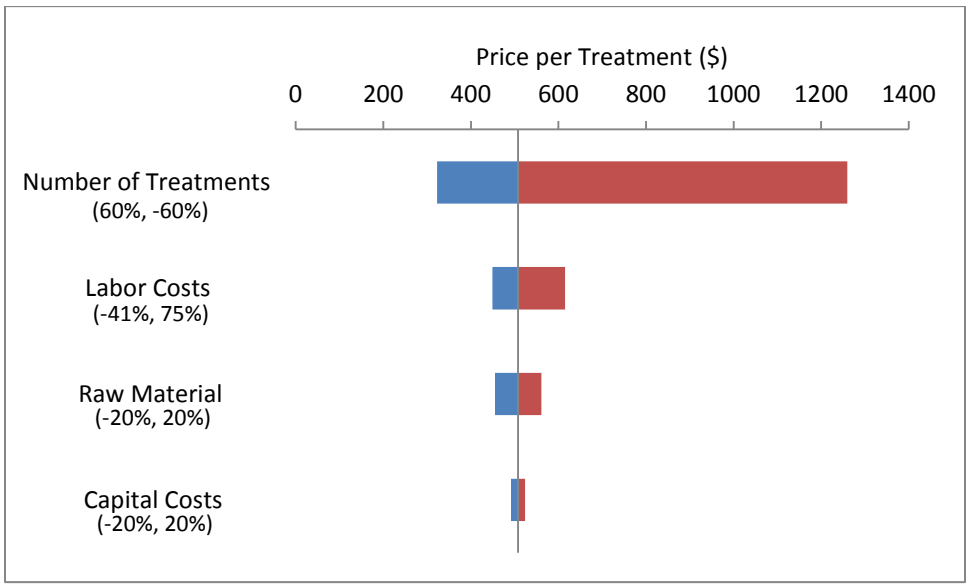

Figure 10: Scenario 3 Tornado Chart

The sensitivity analysis was generated by varying the same four variables in Scenario 1, 2 and 3. The analysis was conducted and the following tornado charts were produced for each scenario as seen in Figure 8: Scenario 1 Tornado Chart**,** Figure 9: Scenario 2 Tornado Chart, and Figure 10: Scenario 3 Tornado Chart. The charts look nearly identical from a qualitative perspective. This makes sense as changes in one scenario should have a similar magnitude of effect in the other scenarios.

The first of these variables is the total amount of treatments produced per run. It was varied from 60% in either direction. The swing in either direction is so large in order to account for inaccuracies that may be discovered in translating research work to manufacturing practice.

The second of these variables is the labor costs. It was varied up to 75% more expensive and 41% less expensive. These percentages were determined from taking the mean annual wage of the top 90th percentile and the 10th percentile for each position[38,39]. Labor costs have the second most effect of all the variables measured.

The third variable is the capital costs. Capital costs were varied by 20% in either direction and found to have the second to least effect of all the variables measured. The 20% movement in either direction is to account for changes in the market and mismatched pricing data collected from the internet when compared with quotes received from companies in detailed design. This analysis would suggest that the price of the capital costs has little effect on the final price of the treatments.

The last variable is the raw material costs. These were varied by 20% in either direction and found to have the least effect of all the variables measured. This analysis would suggest that raw materials cost variability has little effect on the final treatment price.

Monte Carlo Simulation

The Monte Carlo simulation was run using @Risk 7.0 with same variables that were used to develop the tornado charts. All variables were assumed to have a normal distribution, due to the lack of historical data and trends in this sector. The mean of each distribution in the simulation was the original calculated value for that variable. The standard deviation was assumed to be 10% of the original value for all variables except for the number of treatments in which the standard deviation was assumed to be 30% so as to allow for 95% of the values to fall between 60% on either side. The name of the university with the course license has been redacted from the graphs for anonymity.

[38] (Statistics, 2014a)
[39] (Statistics, 2014b)

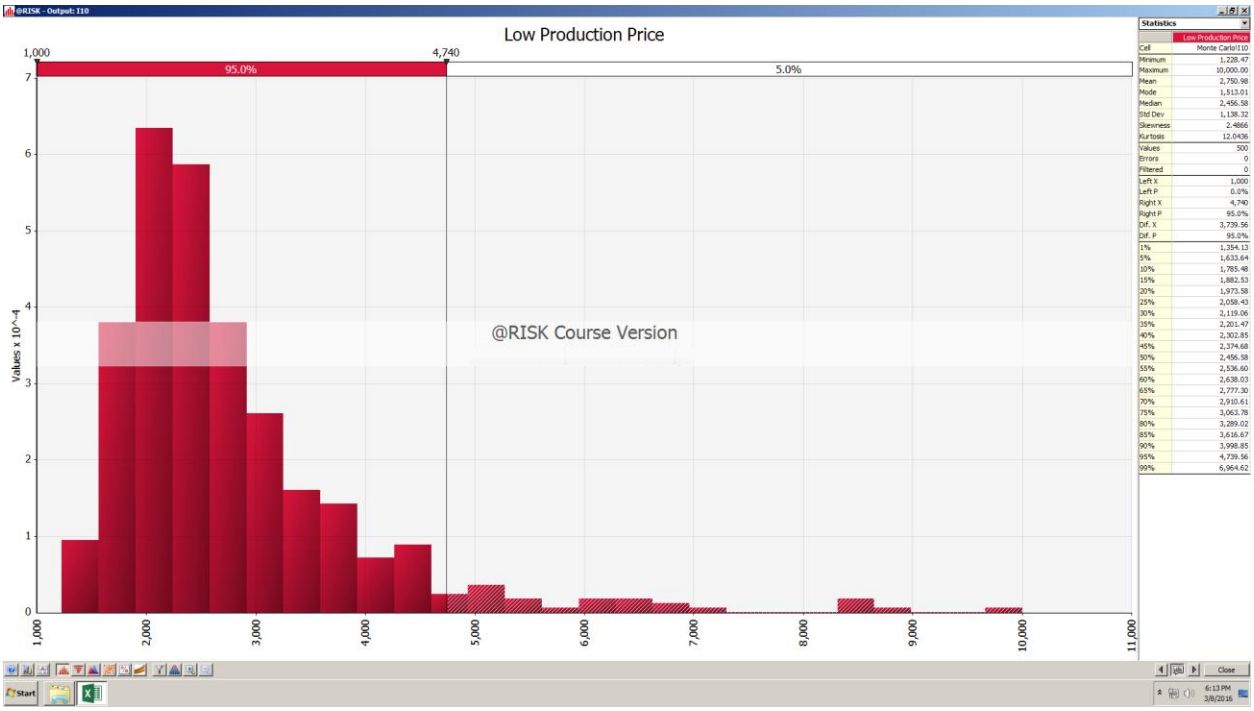

Figure 11: Scenario 1 Monte Carlo Simulation

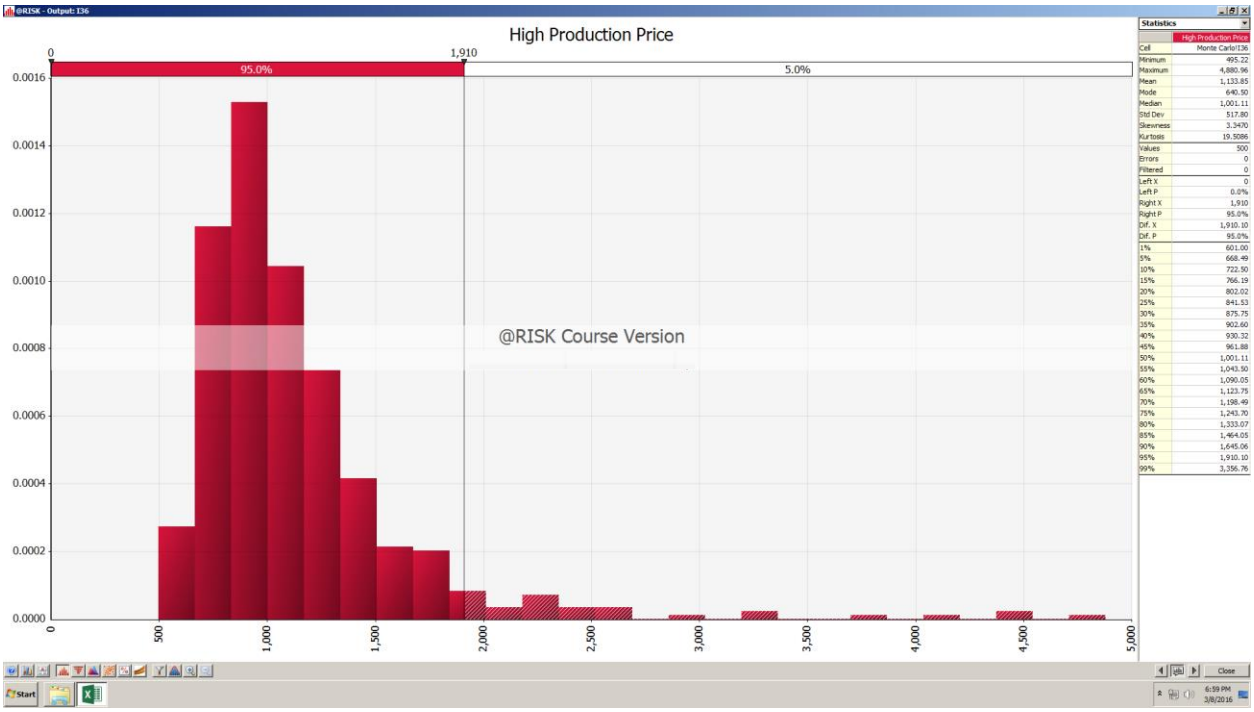

Figure 12: Scenario 2 Monte Carlo Simulation

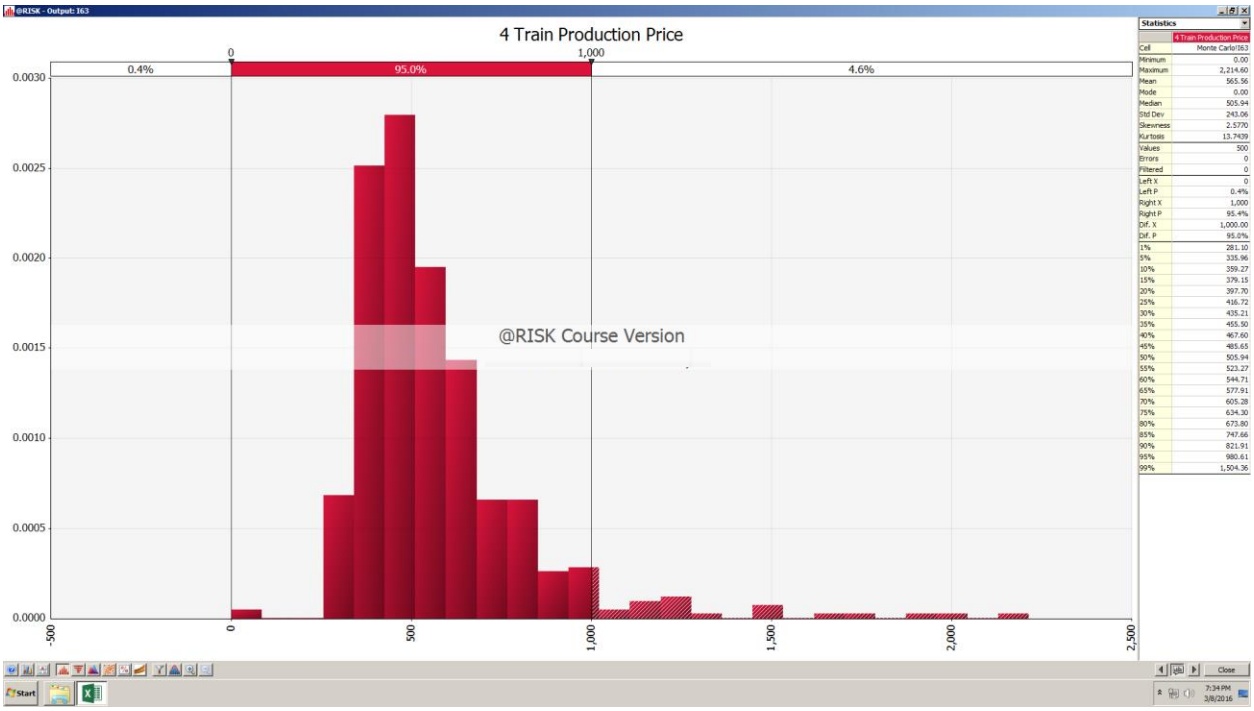

Figure 13: Scenario 3 Monte Carlo Simulation

The Monte Carlo simulation for Scenario 1 can be seen in Figure 11: Scenario 1 Monte Carlo Simulation **,** for Scenario 2 can be seen in Figure 12: Scenario 2 Monte Carlo Simulation and in Scenario 3 can be seen in Figure 13: Scenario 3 Monte Carlo Simulation. An analysis of the simulation shows that as the number of treatments increases the variability of price decreases. This is a result of the increased amount of treatments being sold that attenuates any fluctuations in variable prices.

The prices tend to cluster around the mean price. The Monte Carlo simulation demonstrates that with a 95% of the time the price of the treatment is not more than double the originally calculated price. Finally, the Monte Carlo simulation demonstrates that at all values it projected the cell therapy would still be a superior alternative, both economically and based on quality of life, to traditional spinal cord injury care. This results in very high confidence that the facility will be economically successful.

Other Important Considerations

Virus Inactivation

An important consideration is the inactivation of virus before they enter the manufacturing process. The majority of the media is feeder-free and therefore will be sterile (at least it is assumed to be) upon arrival to the clean room. The exception to this is the neural induction

media. It comes from human plasma and therefore must be irradiated in order to inactivate any potential viruses before its addition to the cellular solutions.

Table 29: Virus Inactivation UV-C Dosage

Virus Inactivation				
Percent Inactivation	Log inactivation	Virus UV dosage (mJ/cm^2)	Require radiation time (min)	44.1
	0.5	39	Power usage (W)	180
90	1	58	Runs/day	4
	1.5	79	Power consumption (kW*hr)	0.53
99	2	100	**Cost of electricy ($/day)**	**0.03**
	2.5	121		
99.9	3	143		
	3.5	163		
99.99	4	186		
99.999	5	226.819		

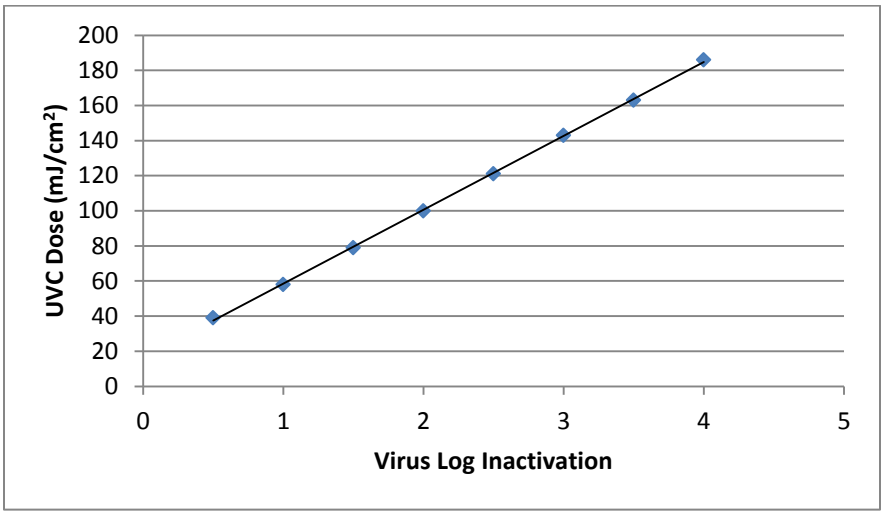

Figure 14: Log Kill of Viruses

The dosage to kill a 99.9% of viruses was inferred from a UV sterilization manufacturer[40]. This dosage was scaled as seen in Table 14 to kill 99.999% of viruses in the media. Based on available literature this dosage is found to be acceptable to treating the media with minimal effect seen in the cells[41].

Timing of Batch Processes

Another important consideration is what the batch process will look like during operation. The Gantt charts provided in Figure 1: Low Production Gantt Chart and Figure 2 offer a good overview of how the equipment is used during the process.

[40] (Solutions, 2016)
[41] (Yen et al., 2014)

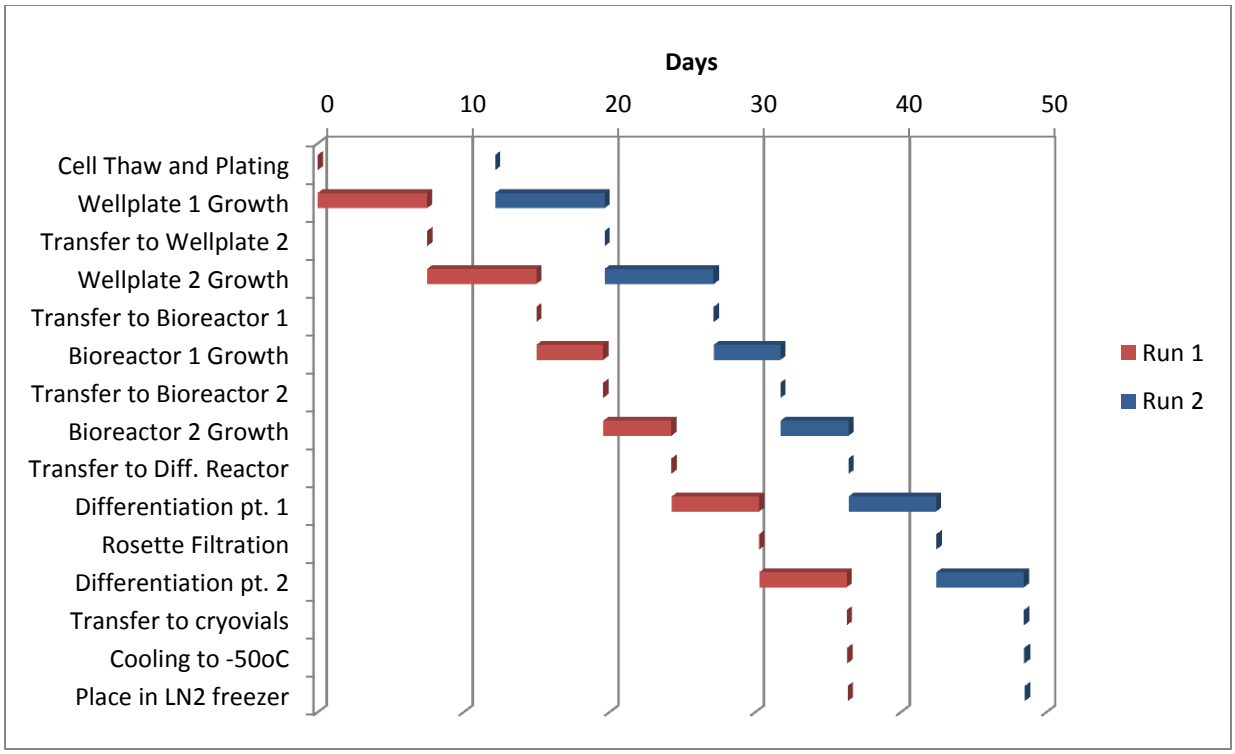

Figure 15: Process Schedule for High Production

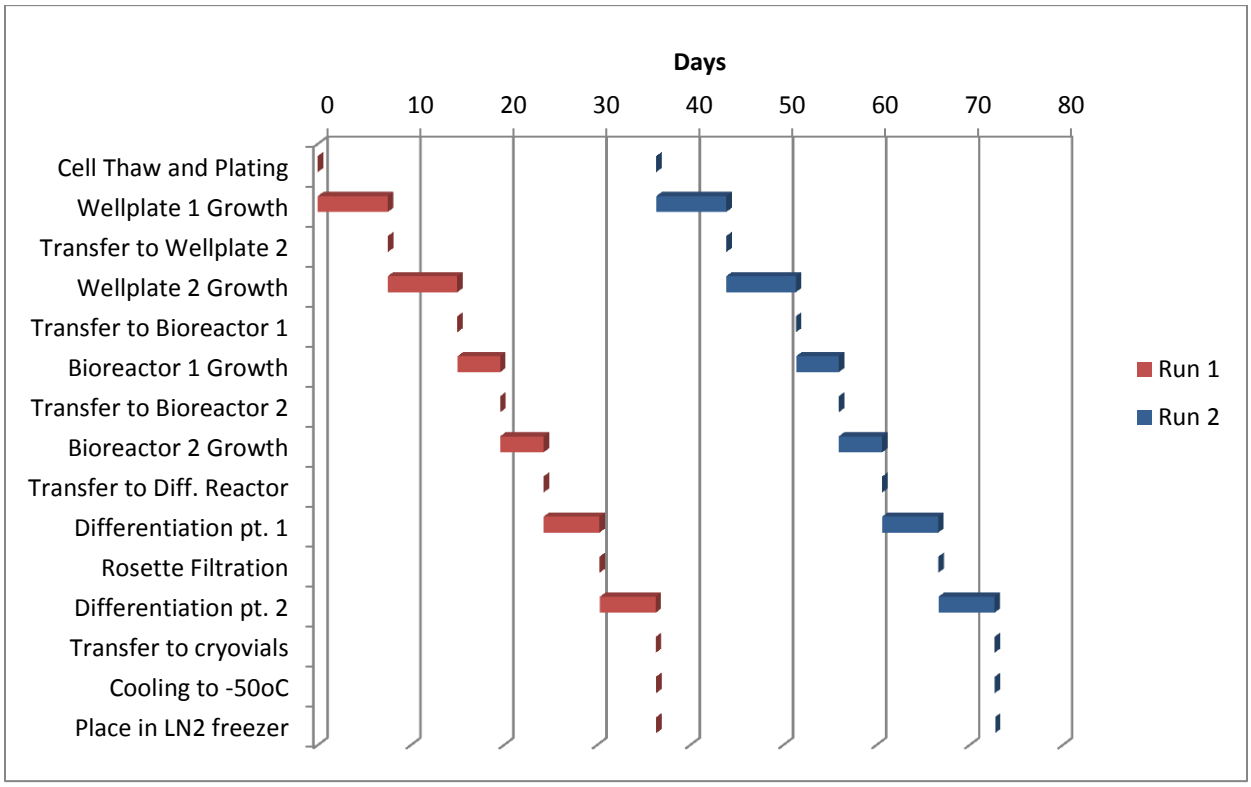

Figure 16: Process Schedule for Low Production

Above in Figure 15 and Figure 16 the process schedule for high and low production is provided. This provides a visually representation of each step that the cells go through.

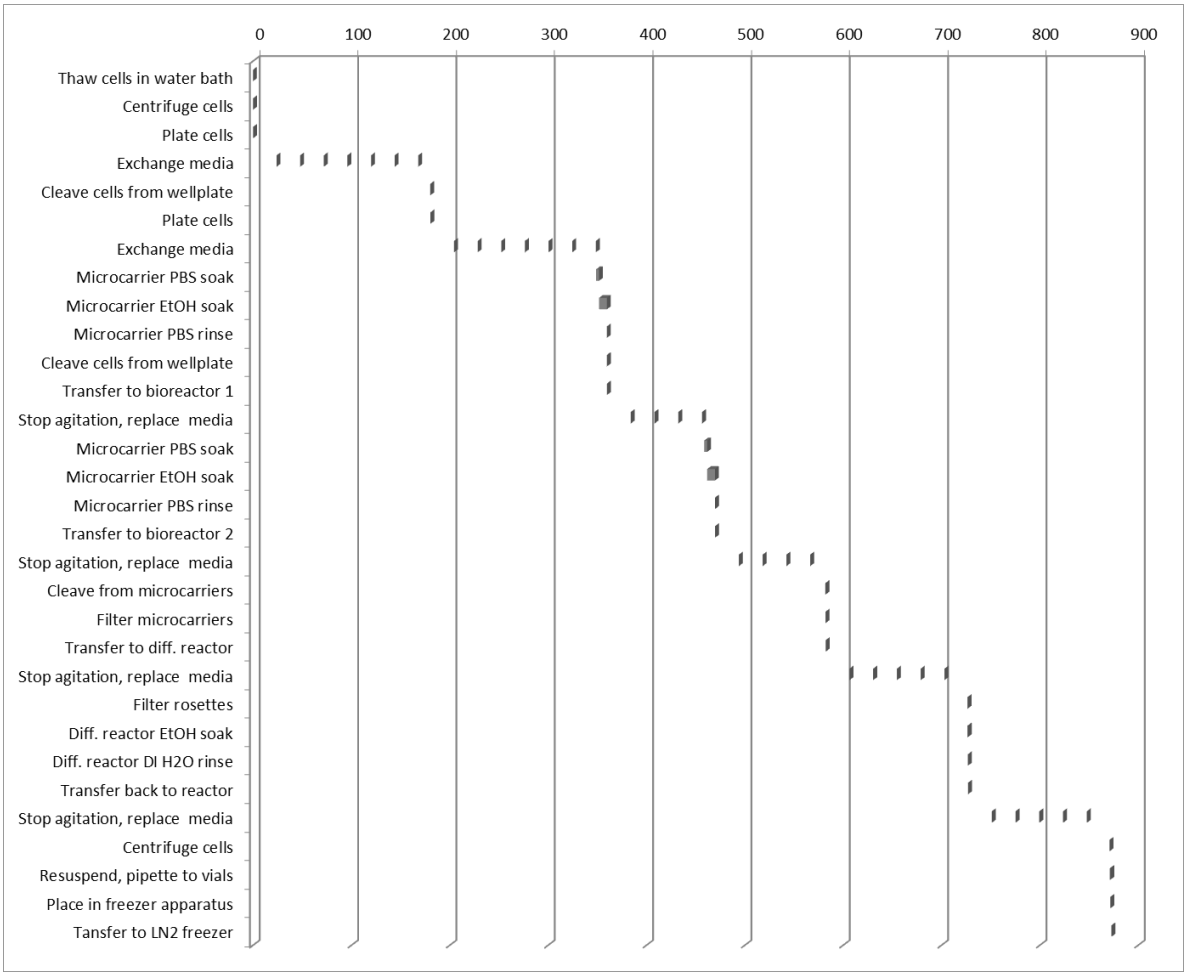

Figure 17: Operation Schedule

In Figure 17 the majority of the steps that a technician would need to go through during a manufacturing campaign are provided. This will look the same for high and low production with regards to a single batch. In high production, the technician will need to attend to other batches in the interim of this schedule.

While significant work has gone into reading, understanding, and verifying the claims (a combination of reasonability coupled with other papers) provided in these papers the exact production levels are certain to change and adaptions to the operation of the process should be a natural occurrence as the company gains experience with this type of design.

Quality Control

Table 30: Quality Control Costs

Item	cost	size	units
Cell counter (QC-1)	$15,000.00	N/A	1
NucleoCassettes	$ 370.00	100	cassettes
Reagent A100	$ 73.50	500	ml
Cell Analyzer (QC-2)	$30,900.00	N/A	1
Wellplate for cell checker - Growth	$ 284.00	1	assay
RNA Kit	$ 4,680.00	400	rxns
RNA Test Wellplate - Diff	$ 284.00	1	assay

Quality control is critical to ensuring that the cells that are sent off as treatment are actually NPCs. In order to make sure that they are this quality control scheme has been developed. First, the QC-1 gives a total cell density and a viable cell density. This allows the facility to know how to adjust if they have too many or too few cells being produced. This will be critical in the beginning stages of the facility as data is being collected to optimize the process. QC-2 is a real time polymerase chain reaction which allows for testing to occur at the end of BR-2. It will first show that the adult stem cells are still iPSCs and haven't yet differentiated. It can also be used to prove that the cells haven't mutated and will not cause cancer in the patients. At the end of BR-3 QC-2 can be used to prove that proper differentiation has occurred.

This process of quality control is more time efficient than the majority of other quality control processes as it allows for results to be determined within the hour as opposed to days or weeks. This allows contaminated batches to be quickly identified and removed and therefore reduces the risks of a bad batch being sent out as well as reducing the costs associated with having a contaminated batch.

Conclusions

This design report outlines the necessary equipment, materials, and operational considerations to operate a stem cell manufacturing facility. Starting with the extensive research into the topic to ensure the best estimations could be provided when making engineering decisions to the rigorous health and safety analysis and protocols this facility has technical merit and is a feasible project to undertake. Furthermore, the operational flexibility afforded to the company due to the unique module design of the facility and its contained bio-reactor trains allow this project to have potential success in many markets. From an economic perspective this project is attractive as all three operational scenarios were able to achieve a 50% IRR while stile producing a treatment at low cost. Even assuming worst case scenarios for the sensitivity analysis the treatment still provided economically attractive results. The treatment's cost is

especially low when compared to the cost of living with spinal cord damage and the quality of life decrease that accompanies a medical disability such as this.

Recommendations

The first recommendation from this project is that it moves on to detailed design. When moving to detailed design special consideration should be given to the protocols and operating procedures the technicians will follow as they offer one of the most successful ways to prevent injury to facility personnel and prevent contamination of the cell batches.

Another recommendation is that the process starts with a single module with one train producing cells. This will allow healthcare professionals and patients to begin testing the effectiveness of this treatment. The company will also be able to gather more data specific to this application to ensure that the assumptions made and values taken from the literature hold true in a larger production context. This also reduces the variability in the number of treatments produced per batch which allows for a more consistent economic analysis to occur because cell treatments produced per batch had the largest effect on the price of the treatment.

Lastly, the company should actively attempt to optimize this process. The optimization should first start with the stem cell growth process and then turn to the differentiation process. As everything develops the company will be able to assume a position in which it can manufacture other types of cell therapies in order to capitalize on their expertise in this technology.

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Appendix A: Energy Calculations

Assumed properties for these calculations:

Media Properties (Based on data for salt water)	
Density (g/mL)	1.02
Heat capacity (J/g*K)	3.99

Cell Properties	
Mass of a cell (kg)	1.00E-12
Density of a cell (g/mL)	1.05
Cell heat capacity (J/g*K)	3.47

N2 Properties	
Density (g/mL)	0.808
dHvap (kJ/mol)	5.56
B.P. (oC)	-195.795

Cell thaw			
One vial		Water bath	
Starting temperature (oC)	-130	Starting water temp (oC)	25
Final temperature (oC)	37	Final water temp (oC)	37
Q (J)	679.71	Heater wattage (W)	360
		Initial heating required (J)	0
		Heat lost to vial (J)	-679.71
		Heat added by heater (J)	679.71
		Time heating (min)	0.03

This calculates how much energy is required to heat the initial vial of cells from their frozen condition to 37°C in a water bath.

Media preheat			
One mL of media		Water bath	
Starting temperature (oC)	25	Starting water temp (oC)	25
Final temperature (oC)	37	Final water temp (oC)	37
Q/m (J/g)	47.88	Heater wattage (W)	360
Q/V (J/mL)	48.84	Initial heating required (J)	501480
		Initial heating time (min)	23.22
		Heat lost to media (J/mL)	-48.84
		Heat added by heater (J/mL)	48.84
		Time heating (min/mL)	0.0023

This calculates how much energy is required to heat media in the water bath. This would be used before the media can be added to the cells in their reactors.

Total Water Bath Usage	
Total Media/batch (mL)	55437
Total Heating time/batch (hr)	2.09
Time preheating bath (hr/year)	141.23
Heater power supply (W)	1032
Energy used/year (kW*hr)	211.35
Cost of energy ($/year)	**$10.57**

This calculates the total amount of energy the water bath will use in a year. This is then used to determine the utility cost of the water bath.

Cell Counter Energy	
Time of test (s)	30
# of tests per batch	4
Test power consumption (W)	25
Standby power consumption (W)	0.002
Running time (hr/year)	1.01
Down-time (hr/year)	8755.94
Total Enegry use (kW*hr/batch)	0.043
Electricty cost ($/year)	$0.00

This is for QC-1. It calculates the amount of energy that it uses over the course of a year. It comes out to less than a penny.

Cell cooling	
One treatment vial	
Starting temperature (oC)	37
Final temperature (oC)	-50
Q (J/vial)	-714.18
Q (J/batch)	-105699.08

This determines how much energy is expended by F-3 to get the treatment down from room temperature to -50°C.

Cell cryopreservation			
One treatment vial		N2 in cooler	
Starting temperature (oC)	-50	Mass evaporated (g/vial)	0.014
Final temperature (oC)	-195	Vol. evaporated (uL/vial)	17.38
Q (J/vial)	-1190.31	Q (J/vial)	2186.54
Qcold+Qhot	9.96E+02	Total mass lost (g/batch)	2.08

This is an energy calculation to determine how much energy is expended by F-4 to get a single treatment vial from -50°C to -195°C.

Electricity Cost ($/kW*hr)	$ 0.05					
Electricity Consumption						
	Voltage (V)	Amperage (A)	Power (W)	Power (kW)	Time running (hrs)	Cost/year
Bioreactor 1 - magnetic stirrer			1050	1.05	8760	$ 459.90
Bioreactor 2	110	3.8	418	0.418	8760	$ 183.08
Differential Reactor	110	3.8	418	0.418	8760	$ 183.08
Fume Hood			261	0.261	8760	$ 114.32
Incubater			460	0.46	8760	$ 201.48
Refrigerator	115	9.4	1081	1.081	2920	$ 157.83
Freezer	115	8	920	0.92	2920	$ 134.32
minus 80 C freezer	115	20	2300	2.3	2920	$ 335.80
UV Irradiator			180	0.18	1073	$ 9.66
UV Cabinet			105	0.105	8760	$ 45.99
Big Centerfuge			260	0.26	1	$ 0.01
Water Bath	120	8.6	1032	1.032	499	$ 25.76
Cell Counter Running			25	0.025	1	$ 0.00
Cell Counter Waiting			0.002	0.000002	8759	$ 0.00
Cell Analyzer			10	0.01	37	$ 0.02
Autoclaver			1400	1.4	46	$ 3.19
					Total	$ 1,854.44

The above table is the calculation for the electricity costs for all of the equipment. The powers were found in the user manuals for the equipment that was used to estimate capital costs. Where power was unable to be found, the voltage and the amperage were multiplied together to approximate the power usage of the equipment. The time for running depends upon the type of equipment. Most capital equipment was running year round and the other energy calculations were used to find the running times for the equipment that did not run year round.

Appendix B: Media and Operating Cost Calculations

Media	Equipment	Volume used (mL)	Microcarriers used (mg)	Growth area (cm^2)	TrypLE Express Used (mL)	Microcarriers used (mg)
2ml	WP1	14.8	n/a	8.8	0.587	-
30ml	WP2	222	n/a	38.4	2.560	-
50ml	BR1	250	400	1080	72.000	0.4
350ml	BR2	1750	2400	6480	432.000	2.4
4L	BR3	24200	n/a	n/a	n/a	-
5L	BR3	29000	n/a	n/a	n/a	-

Microcarriers	
Density (mg/mL)	8
Growth area (cm^2/mg)	2.7

TrypLE Express	
Dosage (mL/cm^2)	0.0667
Price ($)	$ 0.11

The above were used to calculate the amount of media, microcarriers and trypsin used on a per batch basis.

Consumable	Price	Quantity	Unit	Price/quantity	quantity/batch	
Ethanol	$ 111.60	20	L	$ 5.58	9.85	L
WP1	$ 398.10	500	count	$ 0.80	1	count
WP2	$ 255.60	100	count	$ 2.56	1	count
Microcarriers	$ 12,700.00	3000	g	$ 4.23	2.8	g
Microcarrier Prep	$ 27.00	500	ml	$ 0.05	700	ml
40 micron filter - falcon cell strainer	$ 104.00	25	count	$ 4.16	8	count
Falcon 50 mL conical centerfuge tubes	$ 355.00	500	count	$ 0.71	16	count
100 micron filter	$ 104.00	25	count	$ 4.16	8	count
400 micron filter	$ 104.00	25	count	$ 4.16	8	count
cryovials	$ 357.60	500	count	$ 0.72	148	vials
cyropreservation media	$ 205.00	100	ml	$ 2.05	296	ml
Liquid Nitrogen Bath holders/shippers	$ 254.00	1620	vial spots	$ 0.16	148	vials
Growth media	$ 299.00	500	mL	$ 0.60	2236.8	mL
Neural induction media	$ 395.00	500	mL	$ 0.79	24200	mL
Rosette selection media	$ 35.00	100	mL	$ 0.35	29000	mL

This was used to calculate all of the consumables used on a per batch basis.

Per Year Consumption						
Consumable	Price ($)	Quantity	Units	Price/unit ($)	Quantity/day	Price/year ($)
Liquid Nitrogen	$ 232.82	230	L	$ 1.01	0.85	$ 314.05
CO2	$ 28.31	12376	L	$ 0.00	35.3	$ 29.47

This shows the price per quantity for the major chemicals that will be used in the process along with their consumption rates. This was used to estimate the price per year of these chemicals.

Appendix C: Other Utility Costs

The below tables were used to calculate the other utility costs associated with the facility.

Water Cost ($/L)	$ 0.000543			
Water Consumption				
Water Usage	Volume (L/day)	Days	Volume/year (L)	Price/year ($)
Water Bath	10	365	3650	$ 1.98

Water for Injections Cost ($/L)	$ 1.00						
Water for Injection Consumption							
Use	Volume (L)	Units	Yearly usage		Volume (L/year)	Price ($)	
Incubater	5.6775	per week	52	weeks	295.23	$ 295.23	per year
Autoclaver	1.65	per batch	30.41666667	batch/year	50.1875	$ 50.19	per year
Rinsing Sterilization	9.85	per batch				$ 9.85	per batch

Sewage Cost ($/L)	$ 0.00132		
Sewage Use			
Sewage Use	Volume (L)	price	
Water from water bath	3650	$ 4.83	per year
Media After Autoclave	55.4368	$ 0.07	per batch
Water for Autoclave	1.65	$ 0.0022	per batch

Appendix D: Pricing Information

This appendix contains all of the websites that were used to gather pricing information. These prices were on equipment and materials that would be similar to the equipment and materials that would be used in this facility.

Growth media: http://www.stemcell.com/en/Products/Product-Type/Specialized-cell-culture-media/TeSR2.aspx

Neural induction media: http://www.stemcell.com/en/Products/All-Products/STEMdiff-Neural-Induction-Medium.aspx

Rosette selection media: http://www.stemcell.com/en/Products/Product-Type/Specialized-cell-culture-media/STEMdiff-Neural-Rosette-Selection-Reagent.aspx

Ethanol: http://www.carolina.com/specialty-chemicals-d-l/ethanol-70-laboratory-grade-20-l/861265.pr;jsessionid=f9VbeLnDr1Lg4twI0gdRZTQI.worker5?question=

Well plate 1: http://www.fishersci.com/shop/products/thermo-scientific-nunc-dishes-cell-culture-petri-35mm-dish-airvent-500-cs/1256591

Well plate 2: http://www.sigmaaldrich.com/catalog/product/sigma/m8562?lang=en&region=US

Microcarriers:

PBS: http://www.stemcell.com/en/Products/All-Products/DPBS-without-Ca-and-Mg.aspx

40µm filter: https://www.pluriselect.com/cell-strainer.html

50mL centrifuge tubes: https://www.fishersci.com/shop/products/falcon-50ml-conical-centrifuge-tubes-2/p-193321#tab1

100 and 400µm filter: https://www.pluriselect.com/cell-strainer.html

Cryovials: http://www.thermoscientific.com/en/product/nalgene-general-long-term-storage-cryogenic-tubes.html

Cryopreservation media: http://www.stemcell.com/en/Products/Product-Type/Specialized-cell-culture-media/STEMdiff-Neural-Progenitor-Freezing-Medium.aspx

LN_2 storage boxes: http://www.thomassci.com/Laboratory-Supplies/Cryogenic-Storage-Containers/_/Cryoboxes-for-Locator-Systems?q=*

LN_2: http://www.airgas.com/p/NI%20NF230LT22

CO_2: https://www.fishersci.com/shop/products/midi-co-sub-2-sub-incubators/p-4529434

Incubator: https://www.fishersci.com/shop/products/midi-co-sub-2-sub-incubators/p-4529434; https://tuttnauerusa.com/sites/tuttnauerusa.com/files/assets-usa/support/support_MAN_EZ.pdf

Centrifuge: http://www.fishersci.com/shop/orderdisplayview?storeId=10652&langId=-1&shopCart=true&stepNumber=0; http://www.geminibv.nl/labware/heraeus-labofuge-400-4-en?filtertext=&filtercategory=centrifuges-en&filtermanufacturer=

Water bath: http://socalbiomedical.com/equipment/temperature-control-baths/water-baths/general-purpose-digital-water-baths-all-sizes.html?gclid=CNHT1fnTqMsCFQktaQodrFgIXw#page=tab2

Cell counter: https://online-shop.eppendorf.us/US-en/Cell-Manipulation-44522/Accessories-44529/Nucleocounter-NC-100-Cell-Counter-PF-69757.html

PCR analyzer: https://www.thermofisher.com/order/catalog/product/4489084

QC supplies: https://online-shop.eppendorf.us/US-en/Cell-Manipulation-44522/Accessories-44529/Nucleocounter-NC-100-Cell-Counter-PF-69757.html; https://online-shop.eppendorf.us/US-en/Cell-Manipulation-44522/Accessories-44529/Nucleocounter-NC-100-Cell-Counter-PF-69757.html; https://www.thermofisher.com/order/catalog/product/4418722; https://www.thermofisher.com/order/catalog/product/AM1729

-20ºC freezer: http://www.fishersci.com/shop/products/thermo-scientific-revco-high-performance-lab-freezers-automatic-defrost-30-c-11-5cf-115v-60hz/22285655

4ºC fridge: http://www.thermoscientific.com/en/product/revco-high-performance-laboratory-refrigerators-solid-doors.html

Bioreactors: AIChE provided GE Spreadsheet

Magnetic stir plate: http://www.thomassci.com/Equipment/Magnetic-Stirrers/_/7-x-7-Digital-And-Analog-Magnetic-Hotplate-Stirrers?q=*

Fume hood: http://socalbiomedical.com/organization/fume-hoods/captairflowr-laminar-flow-hoods-all-sizes.html?gclid=Cj0KEQiA3t-2BRCKivi-suDY24gBEiQAX1wiXCfLZ805dFvWvGvySc0idk48QD2btw7hYVNzELDUy9saAoKP8P8HAQ

-80ºC freezer: http://www.thermoscientific.com/en/product/80-c-benchtop-freezer.html

In-lab UV cabinet: http://www.csiequipment.com/csi-uv-box-uvb-15_p31597.aspx

Labor: http://www.bls.gov/ooh/life-physical-and-social-science/biological-technicians.htm; http://www.bls.gov/ooh/life-physical-and-social-science/medical-scientists.htm

Autoclave: http://socalbiomedical.com/tuttnauer-ez9-ez10-series-fully-automatic-benchtop-autoclaves.html