Undergraduate Thesis Prospectus

Design for a Novel Recombinant Influenza Vaccine Facility in Brazil

(technical research project in Chemical Engineering)

The Fight for a Better Future in Appalachia

(sociotechnical research project)

by

Michelle Harnisch

November 8, 2024

technical project collaborators:

Abby DeChurch
Mia Holbrook
Diana Kirilov

On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

Michelle Harnisch

Technical advisor: Eric Anderson, Department of Chemical Engineering

STS advisor: Peter Norton, Department of Engineering and Society

General Research Problem

How can underserved populations best be served by external expert institutions?

In the last 125 years, the average life expectancy of a human has more than doubled from 32 to 71 years (Dattani et al., 2023). Increased understanding of medicine, nutrition, and other basic needs has resulted in an individual now being less likely to die at any age than they were in the past (Dattani et al., 2023). Generally, humanity is in better health than it ever was.

However, this phenomenon is not geographically uniform. Wealthier countries, especially in the global north, are more likely to report higher life expectancies due to higher investment in public health (Freeman et al., 2020). Less affluent areas, especially the global south, lag behind (Dattani et al., 2023). Less affluent regions of high-income countries also lag in health indexes. For example, West Virginians in the heart of the Appalachian region of the United States have an average life expectancy 4.2 years lower than that of the average American (Arias et al., 2022). When such disparities are present, how can these communities best be served by the experts around them?

Design for a Novel Recombinant Influenza Vaccine Facility in Brazil

How can the efficient design of a local vaccine manufacturing plant increase production of low-cost and effective vaccines in South America?

Overview

The influenza virus impacts the global population seasonally. According to the Centers for Disease Control, between 100,000-710,000 hospitalizations and 4,900-51,000 flu-related deaths have occurred annually between 2010-2023 in the United States (2024). The flu disproportionately affects at-risk groups including those 65 years and older, children ages 0-5,

pregnant women, and people with underlying conditions. However, even otherwise healthy people can develop serious complications such as sinus infections, pneumonia, or myocarditis if the flu is left untreated.

The H1N1 influenza pandemic of 1918 infected 500 million worldwide and killed between 20 and 50 million. In response, the first flu vaccine was developed in 1945 in the form of an inactivated virus. It was later discovered that multiple strains of the flu exist each year, with the geographic dependence of prevalent strains having been identified by the mid 1950s (WHO, 2024). In the following years, the strains within the inactivated vaccine were adjusted annually, and the vaccines were produced using an egg-based manufacturing method. Despite its effectiveness, this method presented certain disadvantages. People with egg allergies were ineligible for the vaccine. From a manufacturing standpoint, quality control and resource allocation were nonoptimal due to the variability among batches and the work-intensive nature of the process. To combat these challenges, the recombinant flu vaccine was approved by the FDA in 2013 (CDC, 2024). This vaccine is made of influenza antigens rather than inactivated influenza and is the primary vaccine on the market today.

Our team proposes a manufacturing process for a novel recombinant vaccine in a location that has both the resources required to make vaccine production feasible and a demonstrated need for the vaccine. Brazil meets both of these requirements. Brazil has advanced infrastructure and an already established biopharmaceutical manufacturing network through Instituto Butantan. Instituto Butantan is the main producer of influenza vaccines in the Southern Hemisphere, distributing 90 million doses in 2023 (Instituto Butantan, 2024). Given the prevalence of the influenza virus yet low vaccine uptake (21.3% in 2024) (Zeno et al., 2024), there is a demonstrated need for increased vaccine production and access. An independent plant in the

vicinity of Instituto Butantan would have public benefits, removing strain on the large group currently responsible for 65% of vaccines to all Brazillians (UCNTD, 2024), while being able to receive mutual support as biomedical manufacturing facilities. Brazil is also in proximity to multiple Latin American countries with below average vaccination rates such as Paraguay, Peru, and Venezuela (González-Block et al., 2021). The purpose of this facility is to manufacture a competitively priced recombinant influenza vaccine that is accessible to all Latin American populations.

Technical Specifications

The final product of the technical investigation is a design specification for an end-to-end recombinant influenza vaccine manufacturing line in Brazil, serving the Central and South American markets.

The approach of the recombinant flu vaccine is to synthetically reproduce the HA antigen produced by the flu virus. The human body can then recognize anything bearing that antigen and mount an immune response against it, effectively protecting a patient from the flu without them ever coming into contact with the attenuated (weakened) virus. To produce the antigens, host cells are infected with a baculovirus carrying the gene for the desired antigen (CDC, 2022). The basic technology involved is as follows:

1. Host cell line: The *Spodoptera frugiperda* (Sf9) cells are used to host the recombinant baculovirus. The cells are cultured in *Trichoplusia ni* Medium-Formulation Hink (TNM-FH) for optimal nutrients (Thermo Fisher Scientific, 2015). The process is initiated under sterile conditions in a laminar hood, ensuring all external vials are decontaminated with 70% ethanol to maintain sterility. Once thawed from a

cryopreserved stock, the Sf9 cells can be grown adherent in early stages then transferred into suspension culture for scaling up.

2. Batch bioreactor process: cells are grown in controlled batches, infected, and harvested once production is complete. Batch production is preferred for quality control. The Sf9 are grown to approximately 2-4 million cells/mL before infection.
3. Infection of host cells: The influenza vaccine strain, obtained by the CDC, will be cloned using RT-PCR and inserted into a transfer vector containing polyhedrin gene promoters. Co-transfection occurs through the addition of the recombinant baculovirus stock.
4. Infection monitoring and cell analysis: Samples of approximately 4 mL will be collected from each bioreactor at various time points to monitor infection processes at various times, analyzing changes in cell density, viability, and size distribution in an automated cell analyzer. A portion of the sample will be centrifuged and the resulting pellet and supernatant analyzed for SRIF, gel electrophoresis, and blot analysis. The rest of the sample will be used for hemadsorption analysis which measures the protein's ability to bind to red blood cells (RBCs) and indicates successful expression.
5. Antigen purification: In order to isolate the antigen, the mixture will need to be sent through filtration to remove large cell pieces and other contaminants. The product from the filter will then be sent through several chromatography columns utilizing 8 buffers, to ensure the product is pure antigen (Wang et al., 2006).
    a. A combination of tangential flow filtration (TFF) and centrifugation will be used to reduce the cell particulates to concentrate the HA antigen.

b. Chromatography is used for further purification of the antigen suitable for downstream processing. The specific columns used are UNOsphere-Q, SP-Sepharose Fast Flow, and Hydroxyapatite Type I (Wang et al., 2006).
c. Adjuvant Addition: In a Grade A cleanroom environment, the purified HA antigen is mixed with an adjuvant to enhance immune response.

6. Filling and Formulation: the finalized drug substance for each strain is formulated into an ideal dose consisting of two strains of flu type A and two strains of flu type B.
    a. Formulation: The drug substance of four individual strains are aseptically combined into one large batch.
    b. Filling: An automatic vial-filling manufacturing line is contained within a Grade A isolator to fill individual vials with one dose of the formulated drug product.
    c. Lyophilization: The vaccine mixture undergoes freeze-drying to increase shelf-stability.
    d. Inspection: All vials are photographed by a variety of cameras, all searching for a variety of defects in the glass, stopper, cap, or product cake. Rejected vials are removed from the lot. If the number of acceptable vials is within the appropriate threshold, the batch proceeds to storage.
    e. Storage: The vials are held in storage until all appropriate batch records are approved. Once all records have been finalized, the batch will be released for labeling, packaging, and distribution.

Batches will be designated complete after the final step of the process. Samples will be taken along the production line and sent to quality assurance for viral load testing. Once batches have been confirmed to meet regulatory requirements, lyophilized vials will be shipped to an

independent contractor for labeling, packaging, and distribution. The production cycle is approximately 5 days, while the full large-scale manufacturing process is a month.

Execution

This project is overseen by the UVA Department of Chemical Engineering and advised by Professor Eric Anderson. The capstone project as defined by the department is a two-semester series consisting of CHE 4474 and CHE 4476. Abby DeChurch, Michelle Harnisch, Mia Holbrook, and Diana Kirilov are the sole contributors.

The team will meet for one hour a week for check-ins and progress reports. During check-ins, each team member will be responsible for roughly one slide summarizing their findings and future work. Tasks will be assigned at group meetings for individual pursuit, but will be redistributed if needed. Team members are encouraged to seek assistance from faculty and staff members if desired. Other members' presence at these meetings will be strongly encouraged, but never required.

Initially, contributions will be cross-referenced and approved at group meetings. As the team progresses into more technical work in the spring, interdependence on other members' results is expected to grow. It will be the responsibility of each team member to understand and approve the origin for each calculation/decision they inherit from someone else. Likewise, those passing information on should be reasonably certain that their work is correct. It is encouraged that any doubt is voiced as soon as possible to prevent carry through error.

This project exists in the theoretical realm, and will rely heavily on a variety of computational tools. The most relevant one is MATLAB, which will be used for cell growth kinetics and chromatography elution profiles. The supplier of the desired cell line will prove an important resource for determining bioreactor specification (Thermo Fisher Scientific, 2015).

Purification design data will be sourced from existing publications for manufacturing of the recombinant influenza vaccine (Wang et al., 2006).

The Fight for a Better Future in Appalachia

In Appalachia, how are public health proponents, environmental groups, and business groups competing to determine the relative priority of economic opportunity, public health and sustainability in public policy?

Appalachia consists of 420 counties spanning the length of the Appalachian mountains from New York to Georgia (Paskett et al., 2011). Described as "waiting in line for the American dream," Appalachians face a variety of hardships at higher frequencies than their fellow Americans (Clark, 2024). Compared to the national average, Appalachians experience higher rates of unemployment and poverty (Paskett et al., 2011), as well as higher lung cancer mortality, tobacco use, and opioid addiction rates (DeBolt et al., 2021). These issues are attributed as a result of lack of physical infrastructure, geographic isolation, and educational disparities (Paskett et al., 2011; Wies et al., 2020), and represent a pressing problem for a variety of public health, environmental, and business groups. How are these groups organizing to alleviate the problems facing Appalachians today?

Participants include individual community leaders, such as Mayor Cavalier (Whitington et al., 2024) and Youth Community Center Leader John (Wies et al., 2020), role models looking to influence their immediate neighbors by setting positive examples for change. Anne Cavalier was elected mayor of Smithers, West Virginia, in 2018. She has unwavering faith in her community's future, believing that Smithers has "no place to go but up" after the departure of the coal industry left the town at a standstill (Kidd, 2022). Cavalier is working to create a new era of

prosperity for her town by tearing down reminders of the bygone coal industry as well as investing in new solar projects and establishing new community welfare programs (Kidd, 2022; Whitington et al., 2024). John, a youth community center director in Kentucky identified only by his first name, takes a more bottom-up approach. He creates a nurturing environment for the children of his water-insecure hometown, gently encouraging them to opt for the free filtered water provided by the center (Weis et al., 2020). This offers the youth a chance to drink something other than the soda or "unnatural" or "sulfuric" water that John observes is often the only affordable option at home (Weis et al., 2020).

Two types of healthcare providers are also involved, which can be subclassified as: independent primary care physicians concerned about providing adequate care in underfunded areas (Antrim, 2022) and large healthcare conglomerates. One such physician, Dr. Richard Ingram, acknowledges that it is "tough to replicate a multidisciplinary clinic" that can best treat Appalachian cancer patients while simultaneously accounting for "distance and transportation barriers" (Antrim, 2022). He believes that pharmacists are "an untapped resource" and could be essential in closing the gap between patients and healthcare providers in rural Appalachia. On the contrary Ballad Health, a hospital network with a monopoly over much of rural Tennessee and Virginia, claims centralization is the best route to accessible healthcare (Kelman et al., 2024). The CEO of Ballad Health, Alan Levine, argued that healthcare might not even exist in Appalachia without Ballad's intervention, as "the hospitals were on their way to being closed" (Kelman et al., 2024). However, long travel times and extraordinary wait times at Ballad Health hospitals call the viability of this solution into question (Kelman et al., 2024).

Also participating are a variety of advocacy groups. Appalachian Voices, for example, aims to "advance a just transition to a generative and equitable clean energy economy" in order

to “establish economic solutions that create community wealth and sustain Appalachia’s mountains, forests and waters.” (Appalachian Voices, 2024). To them, the future lies in abandoning coal and embracing next generation energy sources. Appalachian Citizens Law Center represents coal miners on the issues of mine safety, black lung, and environmental justice with the goal of protecting “the land and people from misuse and degradation caused by extractive industries” and supporting “a more equitable future in the region” (ACLC, 2024). In addition, Count on Coal, claims to be a “grassroots organization” for coal as the future of the American electrical grid and as a viable avenue for employment (Count on Coal 2024). However, they are managed by the National Mining Association, a trade association whose mission is to “ensure that all aspects of the U.S. mining industry thrive” (NMA, 2022).

Other involved trade groups include the National Association of Tobacco Outlets (NATO, 2024) and the National Association of Convenience Stores (NACS, 2024), who both work to protect the profitability of tobacco products despite tobacco’s negative affect on human health (Antrim, 2022). The National Association of Tobacco Outlets justifies their position because they are defending the “individual rights and the freedom of choice of the adult customers served by tobacco retailers” (NATO, 2024). Likewise, The National Association of Convenience Stores states that “tobacco is a legal product sold responsibly in convenience stores and is a category that is important to the economic viability of the convenience store industry” (NACS). Neither association acknowledges tobacco’s negative health impacts beyond mentioning federal health warnings in passing. The relationship between Appalachians and tobacco is further complicated by the fact that tobacco farming was once a boon for the region, but has all but disappeared in the last two decades (Snell, 2024).

Despite their differing agendas, all participants value prosperity. The discourse lies in how they define it, and how they plan to turn their agendas into tangible change.

References

Antrim, A. (2022, July 19). Patients in Appalachia Face Unique Disparities, Obstacles to Care. *Pharmacy Times*. https://www.pharmacytimes.com/view/patients-in-appalachia-face-unique-disparities-obstacles-to-care

ACLC (2024). Appalachian Citizens' Law Center. (2024). *About Us*. Appalachian Citizens' Law Center. https://aclc.org/about-us/

Appalachian Voices. (2024). *About Us*. Appalachian Voices. https://appvoices.org/about/

Arias, E., Xu, J., Tejada, B., & Murphy, S. L. (2022, August 23). *National Vital Statistics Reports Volume 71, Number 2 August 23, 2022*. CDC stacks. https://www.cdc.gov/nchs/data/nvsr/nvsr71/nvsr71-02.pdf

CDC (2022). Centers for Disease Control. (2022, November 3). *How Influenza (Flu) Vaccines Are Made | Influenza (Flu)*. CDC. https://www.cdc.gov/flu/vaccine-process/?CDC_AAref_Val=https://www.cdc.gov/flu/prevent/how-fluvaccine-made.htm

CDC (2024). Centers for Disease Control. (2024, August 2). *About Estimated Flu Burden | Flu Burden*. CDC. https://www.cdc.gov/flu-burden/php/about/index.html

CDC (2024). Centers for Disease Control. (2024, September 9). *Recombinant Influenza (Flu) Vaccine | Influenza (Flu)*. CDC. https://www.cdc.gov/flu/vaccine-types/flublok-vaccine.html

Clark, D. B. (2024, September 10). Book Review: 'Stolen Pride,' by Arlie Russell Hochschild. *The New York Times*. https://www.nytimes.com/2024/09/10/books/review/stolen-pride-arlie-russell-hochschild.html

Count on Coal. (2024). *About Us – Count on Coal – Reliable. Affordable. American*. Count on Coal. https://www.countoncoal.org/about-us/

Dattani, S., Rodés-Guirao, L., Ritchie, H., Ortiz-Ospina, E., & Roser, M. (2023). *Life Expectancy*. Our World in Data. https://ourworldindata.org/life-expectancy#all-charts%5C

DeBolt, C. L., Brizendine, C., Tomann, M. M., & Harris, D. A. (2021, Sep.). Lung Disease in Central Appalachia: It's More than Coal Dust that Drives Disparities. *Yale Journal of Biology and Medicine*, *94*(3), 477-486. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8461577/

Freeman, T., Gesesew, H. A., Bambra, C., Justo Giugliani, E. R., Popay, J., Sanders, D., Macinko, J., Musolino, C., & Baum, F. (2020, November 10). Why do some countries do better or worse in life expectancy relative to income? An analysis of Brazil, Ethiopia, and the United States of America. *International Journal for Equity in Health*, *19*(202). https://doi.org/10.1186/s12939-020-01315-z

González-Block, M. Á., Pelcastre-Villafuerte, B. E., Knauth, D. R., Fachel-Leal, A., Comes, Y., Crocco, P., Noboa, L., Zea, B. R., Ruoti, M., Díaz Portillo, S. P., & Sarti, E. (2021, August 12). Influenza vaccination hesitancy in large urban centers in South America. Qualitative analysis of confidence, complacency and convenience across risk groups. *PLOS ONE*, *16*(8). https://doi.org/10.1371/journal.pone.0256040

Instituto Butantan. (2024). *Instituto Butantan - The Institute*. Butantan Institute. https://en.butantan.gov.br/institute.php

Kelman, B., & Liss, S. (2024, March 25). After Appalachian hospitals merged into a monopoly, their ERs slowed to a crawl. *Fierce Healthcare*. https://www.fiercehealthcare.com/providers/after-appalachian-hospitals-merged-monopoly-their-ers-slowed-crawl

Kidd, D. (2022, June 1). *Possibilitarian Anne Cavalier is firing up Smithers*. WV Living Magazine. https://wvliving.com/possibilitarian-anne-cavalier/

NACS (2024). National Association of Convenience Stores. (2024). *Advocacy: Tobacco*. NACS. https://www.convenience.org/Advocacy/Issues/Tobacco

NATO (2024). National Association of Tobacco Outlets. (2024). *NATO: About - Mission Statement*. National Association of Tobacco Outlets. https://www.natocentral.org/about/mission

National Mining Association. (2022). *National Mining Association Membership*. National Mining Association. https://nma.org/about-nma-2/membership/

Paskett, E. D., Fisher, J. L., Lengerich, E. J., Schoenberg, N. E., Kennedy, S. K., Conn, M. E., Roberto, K. A., Dwyer, S. K., Fickle, D., & Dignan, M. (2011, July 12). Disparities in Underserved White Populations: The Case of Cancer‐Related Disparities in Appalachia. *The Oncologist*, *16*(8), 1072–1081. Web of Science. https://doi.org/10.1634/theoncologist.2011-0145

Snell, W. (2024, March 27). *Census Reveals Tobacco Farms Disappearing from Southern Agriculture*. Southern Ag Today. https://southernagtoday.org/2024/03/27/census-reveals-tobacco-farms-disappearing-from-southern-agriculture/

Thermo Fisher Scientific. (2015, April 21). *Growth and maintenance of insect cell lines User Guide (Pub.No. MAN0000030 C.0)*. Thermo Fisher Scientific. https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Insect_Cell_Lines_UG.pdf

UCNTD (2024). Uniting to Combat Neglected Tropical Diseases. (2024). *Butantan Institute | Uniting to Combat NTDs partner*. Uniting to Combat NTDs. https://unitingtocombatntds.org/en/about-us/partners/butantan-institute/

Wang, K., Holtz, K. M., Anderson, K., Chubet, R., Mahmoud, W., & Cox, M. M.J. (2006, March 15). Expression and Purification of an Influenza Hemagglutinin—One Step Closer to a Recombinant Protein-Based Influenza Vaccine. *Vaccine*, *24*(12), 2176-2185. https://doi.org/10.1016/j.vaccine.2005.11.005

Whitington, J., & Oguz, Z. (2024, Sep.). Mining the Sun: Coal-to-solar transitions and energetic place-making in Appalachia. *Critique of Anthropology*, *44*(3), 276-293. Web of Science. https://doi.org/10.1177/0308275X241269614

Wies, J. R., Mays, A., Collins, S. M., & Young, S. L. (2020, July 27). “As Long As We Have the Mine, We'll Have Water”: Exploring Water Insecurity in Appalachia. *Annals of Anthropological Practice*, *44*, 65-76. Web of Science. https://doi.org/10.1111/napa.12134

WHO (2024). World Health Organization. (2024). *History of Influenza Vaccination*. World Health Organization (WHO). https://www.who.int/news-room/spotlight/history-of-vaccination/history-of-influenza-vaccination

Zeno, E. E., Nogareda, F., Regan, A., et al. (2024, October 3). Interim effectiveness estimates of 2024 Southern Hemisphere influenza vaccines in preventing influenza-associated hospitalization—REVELAC-i Network, Five South American Countries, March–July 2024. MMWR Morbidity and Mortality Weekly Report, 73, 861–868. http://dx.doi.org/10.15585/mmwr.mm7339a1