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1. Summary

SenseNC has developed a biosensor that integrates peptide chains into a molecularly imprinted polymer (MIP) matrix in tandem with peptide affinity to detect vancomycin in blood plasma. This “dual recognition” technique is hopeful for providing a synergistic improvement of biosensor sensitivity and selectivity. The electronic element of the biosensor governs the measurement of vancomycin present by the change in resistance to electrical current as vancomycin occupies the pockets in the MIP through electrochemical impedance spectroscopy (EIS). Our device offers several advantages, including small size and ease of use, which allow healthcare providers to test for vancomycin at a patient’s bedside, thereby eliminating the need to send patient samples to a clinical laboratory. After a thorough investigation of the United States healthcare market through interviews and secondary research, SenseNC has created a device to solve the pains of patient suffering and hospital losses.

2. Biosensor System and Assay

We have developed an electrochemical biosensor for the quantification of vancomycin concentration in human plasma using a molecularly imprinted polymer (MIP) containing an additional peptide-based recognition element on a gold interdigitated electrode array.

2.1 Molecularly Imprinted Polymers (MIP)

A MIP is a polymer that is synthesized around a target molecule [1]. Prior to polymerization, a template molecule is added to the monomer solution. Upon completion of the polymerization process, the target molecule, which does not participate in the polymerization process, is removed with an elution technique. This leaves behind pockets in the polymer matrix that possess steric and chemical specificity to the template, much like a "lock and key" concept as depicted in **Figure 1**. In our biosensor, the MIP acts as the biorecognition element by capturing vancomycin.
Various monomers in the MIPs have specific functions. N-isopropylacrylamide (NIPAm) acts as the backbone monomer. N-N'-Methylenebisacrylamide (BIS) acts as crosslinker. N-tert-Butylacrylamide (tBAM) and acrylic acid (AA) act as the hydrophobic and hydrophilic functional monomers, respectively. Functional monomers have affinity to moieties on a template molecule (i.e. vancomycin). We derived the formula for our MIPs from a previous formulation developed by Poma et al. using the same backbone, crosslinker and functional monomers [2].

The MIP is formed by reversible addition-fragmentation chain transfer (RAFT) polymerization. The advantage that the RAFT mechanism has over conventional free radical polymerization is the ability to control the extent of reaction.

This difference can be traced to the RAFT polymerization agent in our sensor: the iniferter. Iniferter is a compound term for "initiator-chain transfer agent-terminator. Unlike traditional photoinitiators, iniferters form active and dormant free-radical "halves" that conduct polymerization during UV irradiation, but cease the reaction after the UV light is removed. This is how the thickness of our film can be controlled and replicated. In our biosensor, the polymer film is built from the surface of the working electrode using a thiol-functionalized diethyldithiocarbamate iniferter molecule. The synthesis and molecular structure of the iniferter is depicted in **Figure 2** [3]**.**

2.2 Peptide Affinity Binding

Just as an antibody has specificity for an antigen, a peptide can possess a specific noncovalent interaction for another molecule, albeit with lower affinity. The mechanism of action of vancomycin involves recognizing and binding to an amino acid sequence, Lysine-d-alanine-d-alanine (Kaa), that is present on the cell wall of gram positive bacteria [2]. Based on this natural action of vancomycin, we have synthesized an allyl-modified form of the Kaa peptide sequence to integrate within the molecularly imprinted polymer. The allyl modification allows for the peptide to participate in the polymerization and behave as a functional monomer. We hypothesize that the incorporation of this peptide recognition sequence improves the specificity of the biosensor. The specific attractions between vancomycin and Kaa are depicted in **Figure 3**.

Figure 1. Representation of the "Lock and Key" MIP analogy.

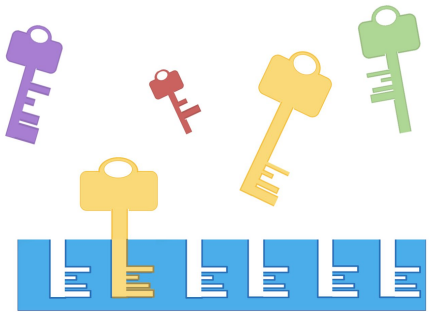
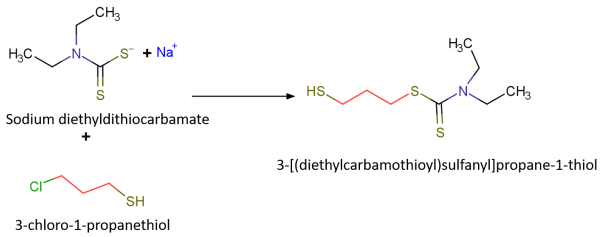


Figure 2. Iniferter synthesis pathway.



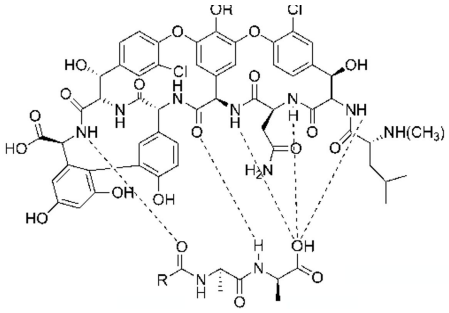


Figure 3. The D-alanine residues of the peptide exhibit five hydrogen bonding interactions with vancomycin [4].

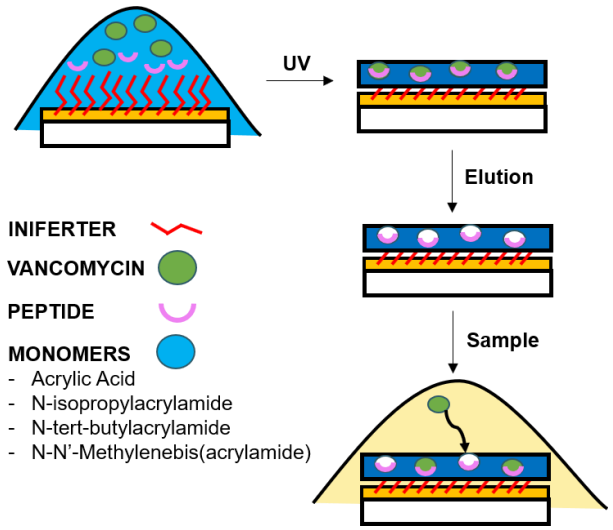
2.3 Dual Recognition

By integrating the peptide into the monomer solution used to form the MIP, the peptide acts as an additional functional monomer to further enhance the affinity and specificity of the MIP for vancomycin. Thus, vancomycin should be detectable at lower concentrations. During MIP formation, the peptide likely bonds near vancomycin and remains in the pocket formed after elution as shown in **Figure 4**. SenseNC has coined this in tandem detection sensor as a "Dual Recognition" element.

2.4 Electrochemical Detection

During the testing process, the vancomycin sample matrix is injected into the sensor device.

Figure 4. MIP formation and elution pathway.



As the sample flows across the polymer, vancomycin in the sample occupies pockets within the MIP. The occupation of vancomycin in these pockets corresponds to different impedance measurements determined through electrochemical impedance spectroscopy (EIS) [5].

EIS is a technique of determining electrical impedance ($z(\omega)$) by applying a small potential range (1-10 mV), alternating current (AC) signal, and measuring the phase shift (ϕ) and amplitude change (z_0) of the resulting output potential. The change in impedance can be reported based on the relationship given below:

$$z(\omega) = z_0(\cos\phi + j\sin\phi)$$

By applying a large range of different frequencies, a specific frequency can be identified that demonstrates the most distinct change in impedance as the vancomycin interacts with the surface of the sensor. The impedance across a MIP containing vancomycin is expected to be higher than a MIP with empty cavities, and the phase shift in that signal difference can be correlated back to a measured concentration value.

3. Analytical Performance

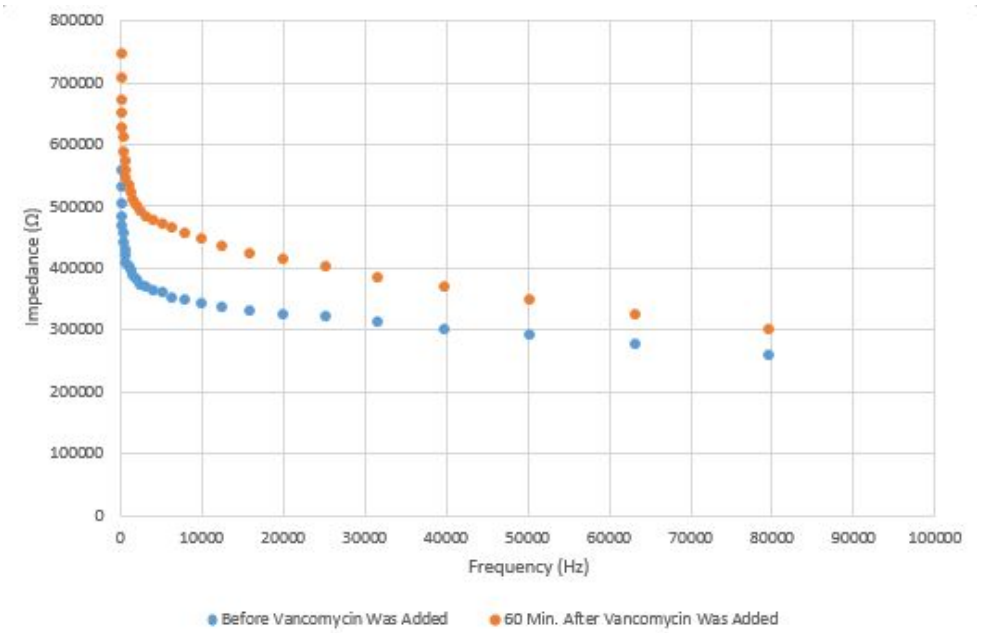
3.1 Analytical Design

The fabrication of the MIP synthesis was The sensor works by placing the functionalized electrode in an electrochemical cell with an aqueous vancomycin sample, a reference electrode, and a counter electrode to take EIS measurements for concentration.

3.2 Preliminary Biosensensor Tests

Optimal parameters for polymerization first had to be established. Initially, a polymer film was not observed during fabrication. It was determined that the structure of the chosen surface linker was not conducive to stimulate free radical polymerization so the surface linker choice was changed from 4-chlorobenzenemethanethiol to 3-chloro-1-propanethiol. Then, the new iniferter and monomer solution were prepared separately in a nitrogen atmosphere. Gold interdigitated electrodes were cleaned then incubated in iniferter in a nitrogen environment for 24 hours. Finally, the monomer solution and vancomycin template were added to the surface of the chip just before UV exposure under nitrogen. Prior to introducing the template molecule to form a MIP, this system was tested by serial polymerizations on blank gold wafers to ensure film formation with different monomer solution formulations. To achieve uniform film thickness, once proper iniferter and polymer solutions were calculated and synthesized, multiple trials were performed to optimize consistent film formation and thickness. These experiments included varying UV light exposure times, altering the setup of the UV equipment to monitor film formation, and a positive control in the form of a photoinitiator to ensure monomer solution was functional. The optimal UV exposure time used for polymerization was determined to be 30 minutes. Film hydrophilicity and thickness were measured by contact angle goniometry and profilometry, respectively. Once the MIP was polymerized, the template molecule was eluted off the film with an aqueous buffer solution. EIS has been and continues to be run in order to validate the elution behavior, the subsequent reintroduction of vancomycin to the imprinted polymer, and the associated response related to these behaviors. An example of this validation testing is shown below, in **Figure 5**.

Figure 5. Validation of template elution by EIS.



4. Novelty and Creativity

4.1 Already Available

Monitoring of active vancomycin levels in hospital patients is currently done by lab-based assays executed by technicians and interpreted by doctors. There is a present need for a faster, reliable, user-friendly detection method for vancomycin in order to improve the efficacy of vancomycin treatment.

MIPs are widely implemented in the biosensing industry because they have high bio-recognition capability and chemical stability. MIPs are also easy to prepare and operate at a low cost [6]. To create the recognition element of the MIP, cavities of the imprinted target cells must be assayed, sized, and shaped to identify the target molecule [6]. MIPs have been used successfully in many fields of biosensing, including registering the presence of microorganisms in a liquid, detection or screening of drugs, environmental analysis, and clinical diagnosis [7].

In our laboratories at NCSU, we were able to take advantage of many already available technologies, including electrochemical analysis tools, cleanroom microfabrication and verification equipment, and circuit design, rework studios, and state of the art synthesis equipment.

4.2 New Developments

For the chemistry, SenseNC revisits the idea of using molecularly imprinted polymers (MIPs) as a base for detection. Although this is a commonly used method for electrochemical analysis, SenseNC focused on the creation of a thin film, rather than the conventional nanoparticles used for vancomycin detection. It is suggested from preliminary testing that a larger MIP film will perform to the same degree of sensitivity and selectivity. SenseNC is also studying the use of peptides as monomers for the MIPs because there has been minimal previous research into the feasibility of photopolymerized peptide monomers. The main source of technical novelty lies in this “dual-recognition” element built into every cartridge. The in-tandem detection with both MIP and peptide was not found in any existing literature after extensive review. This expected edge in sensor specificity and sensitivity stands as a model for future biosensors waiting to make it on the healthcare market.

The chemistry and electronic skills SenseNC bring to the table offer a unique advantage and solution to the current vancomycin analysis system. The technique SenseNC offers will cut down on time as well as cost, as the process will move towards modularity rather than a system of travel between hospitals and labs. This technology will allow for the use and diagnosis of vancomycin by a patients bedside.

5. Translation Potential

5.1 Stakeholder Desirability

With the demand for point-of-care testing on the rise, the healthcare industry is need of up-to-date technology that can quicken the data analysis process. Therefore, through the adoption of handheld biosensors into the industry, there will be improvements in the real time continuity of care. The unique value proposition of the SenseNC biosensor lies within the mobility design, the modularity process and the usability. The problem with the current process is the high risk associated with misdosing Vancomycin due to lack of infrequent measurement intervals (1-3 days). We interviewed a local hospital manager, and every day a patient stays in the hospital, their risk to complications from Vancomycin treatment goes up by 2%.

To fully understand the medical needs around the usage of vancomycin, and, in larger respect, the needs of patients inside hospitals fighting life threatening infections, our team recognized early on that it would be critical to gain first hand customer feedback from decision makers and stakeholders inside of hospitals. Through multiple in-person interviews, which took place inside of local hospitals with physicians and clinical directors, and questionnaires emailed to nurses and medical students, our team gained valuable insight into the realities of healthcare providers. Through these interviews, our team learned that current hospital procedure requires the analysis of vancomycin levels at fixed intervals. Since vancomycin effectiveness is based on dose maintenance levels not increased amounts, it is critical for patient blood samples to be drawn at the correct time point and to have the results analyzed quickly. However, general hospital procedure in the United States involves drawing a patient blood sample, then sending that sample down to a clinical laboratory housed either within the hospital or a nearby laboratory corporation for analysis. With the adoption of the SenseNC Biosensor, physicians will be able to test vancomycin levels on site close to the point of care. According to an interview the team conducted, 30% of patients have kidney complications due to inaccurate regulation of Vancomycin levels. The use of a biosensor that gives faster results would help mitigate this risk.

Our team met with a Pharmacist and an ER Manager from Rex Hospital. They provided us with information on some desired measurement features for a biosensor product for hospital use. Our team highly valued this information, as customer centered design is key in medical products. From our in customer interviews, our team gained the following high level product requirements:

- That the total blood sample size be small (50uL, or less, including any headspace needed), specifically so our solution would be ideal for pediatric and infant blood measuring. It is ideal to draw as little blood as possible from this patient population. (Steven W. Cotten PhD DABCC, Co-Director of Clinical Chemistry)
- The device be portable, and operated by a nurse that is close to the patient population.(Marty Cooney, Infection Prevention Manager, UNC Healthcare).
- Our device communicate wireless with hospital medical record systems, to insure patient data is not mixed up and accessible immediately(Sarah, UNC Nurse Manager).
- Our device have user input screens that identify the patient, administering nurse, and test strip ID before levels are obtained. This fits with current clinical practice with devices such as blood glucose monitors (Sara, UNC Nurse Manager).

From the standpoint of stakeholders outside of those in the direct path of care(patient, doctors, nurses), insurance companies that are currently liable financially for the cost incurred during extended hospital stays due to the current measurement intervals of vancomycin measurements will benefit. Point of care sensing will allow for hospital staff to more accurately maintain vancomycin dose levels, which will lead to less treatment complications and shorter patient hospital stays, both of which equate to less hospital billing cost that insurance companies are liable for currently.

5.2 Technical Feasibility

Nurses at UNC Medical Center identified a hand-held device for measuring blood glucose levels to be key for ease and hasteness of communication and consistency among data. Similarly, at least from an electronic standpoint, our vancomycin monitoring device could be a small

hand held device not much bigger than a cell phone as seen in **Figure 6**. A fully custom PCB with microcontroller, wireless communication and our impedance analysing circuit is all that is inhibiting our device from being much smaller than the current prototype. The electrical components used are extremely small (mm scale). In comparison, current hospital equipment to measure vancomycin levels is large industrial scale machinery which is housed in its own dedicated room.

The goal of a similar user experience to current handheld hospital devices directly shaped the user experience (UI) of our device. In order for our device to be uptaken by the medical market, it needs to fit into the current clinical workflow and procedures of hospital staff. From specific feedback gained from hospital nursing staff, we designed the UI. The nurse will register the patient, themselves, and the test strip identifier into the device before testing is conducted. After testing of vancomycin levels is conducted, the level will clearly be shown on the screen of the device. The handheld vancomycin sensor will then upload patient test data wirelessly to the hospitals patient record systems.

We envision that production ready casing and electronic designs can be ready for manufacture within a 4-6 month period, assuming a small team of full time engineerings are employed. After a product prototype has been developed and verified in hour, the next step in the business would be to seek federal approval to use our device as a medical diagnostic tool. In the USA, approval from the Federal Drug Administration (FDA) would need to be gained. 510K approval, or “looks like”, approval would be sought, as the approval cost is drastically lower (~300,000 USD) compared to a full pre-market approval (PMA) process (~500,000 to 2,000, 000 USD).

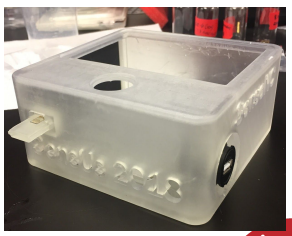
5.3 Business Viability

The main financial benefit of our solution comes from the point of care feature that allows for more frequent vancomycin level readings in patients. When hospitals experience a case of AKI (Acute Kidney Injury) due to improper vancomycin dosing, the added cost of care that the hospital must absorb is estimated at $8,000 or about €6.480 per patient per AKI incident (Source: UNC Hospital in person interview). According to interviews with a local hospital infection control manager, the vast majority of these AKI cases are due to current procedures that do not monitor vancomycin frequently enough, or relay on nursing staff to monitor levels on very strict intervals. Currently, when liability due to AKI cases, assay level reading in lab, and drug cost are summed, a local hospital quoted us an approximately sum cost $196/ €167 per day per patient for vancomycin treatment. The largest part of this aggregated daily cost is the AKI liability cost, at $160/€136 per day. Reducing the risk of AKI through frequent patient level measurements is the main financial cost saving case for the hospital.

The second highest cost in vancomycin treatment comes from the hospital staff time. Currently, the overall patient cost is approximately $7,000 for vancomycin infection treatment. The cost of the actual vancomycin drug is relatively small, our hospital source quoted us a hospital incurred cost of $10.72/€9.17 per day for the drug. A large proportion of this $7,000/€6,000 cost of vancomycin treatment cost is in the staff time required to monitor the administering of the drug. Our solution, which will cut out a portion of the personnel cost of measuring vancomycin away from the point of care, will reduce the overall personal efforts, specifically lab technician time, involved in currently measuring patient vancomycin levels.

Our technology offers a source of constant revenue as the device itself is reusable but the individual cartridges are single use. The business process would mirror the Razer Blade Model, where the analysing device is sold for close to cost and revenue is achieved primarily through the sale of the single use MIP sensing chips. From a device cost standpoint, our BOM estimates for quantities in the 1-10K range estimate that the device casing, LCD touch screen, PCB board, microcontroller, and supporting electrical circuitry will come to approximately $90-$110 per analysing unit. The part cost of the manufactured MIP electrodes is estimated to be in the $3-$8 range at 10,000+ quantities.

Figure 6. 3D model of finished design (Left) and first printed prototype (Right).



6. Team and Support

6.1 Contributions of the team members

Madisen Andersen, Business Administration Undergraduate, designed promotional material and spoke with the College of Management to gain sponsorship, assisted writing translational portion.
Alice Di Fazio, Chemical Engineering Undergraduate, researched methods of vancomycin detection, conducted polymerization testing and device development, assisted writing the Summary and Analytical Performance sections of FRD.
Hannah Johnson, Materials Science Undergraduate, designed promotional material and spoke with sponsors. She also wrote the Novelty and Creativity section of FRD, assisted in writing the translational pitch, and ordered team uniforms.
Chris Fesmire, Biomedical Engineering Graduate, conducted in person customer research. Chris designed the prototype and user experience around the customer comments and requirements recieved, and assisted with electrical design.
Sydney Floryanzia, Chemical Engineering Undergraduate, researched methods and current approaches for vancomycin detection, worked on cleaning and polymerizing of gold chips, wrote part of Biosensor System and Assay section, and delivered public and 1 min pitches.
Dhruvi Fulfagar, Industrial Design Graduate, conducted custom research and produced design sketches and ideation from the customer feedback.
Chuck Geddie, Biomedical Engineering Graduate, conducted in-person user experience research at two university hospitals, researched possible device configurations, designed and optimized protocol to functionalize electrodes.
Matt Sabo, Electrical Engineering Undergraduate, programmed the device, designed the LCD graphics, developed app application, and built the website and web backend.
Mike Wilkins, Electrical Engineering Graduate, worked on programming, designed electrical circuit, conducted electrochemical testing, developed microfluidics, spoke with sponsors, interviewed orthopedists.
Calvin Shanahan, Chemical Engineering Undergraduate, worked as the team liaison with the SensUs organization, organized meetings, researched and executed chemical experiments and protocols for electrode functionalization and validation.
Brendan Turner, Biomedical Engineering Graduate student, initially researched possible device configurations for vancomycin detection. Developed, tested, and implemented interface between gold electrode and MIP, performed troubleshooting during device development, and tested device parameters.
Kristina Rivera (coach), Biomedical Engineering Graduate, created sponsorship letters and spoke with sponsors, organized documents and deliverables, and conducted interviews with UNC Chapel Hill hospital doctors, nurses, and medical students.

6.2 People who have given support

We received generous support from the hospital administrative and nursing staff at UNC REX and UNC Chapel Hill hospitals, respectively. They allowed us to conduct numerous interviews and tour the laboratory facilities where hospital assays are performed on a large scale. They discussed potential desire to collaborate and provide human samples with known concentrations of vancomycin to compare measurements with the biosensor.

6.3 Sponsors

The NCSU College of Engineering's Dean of Academic Affairs, Dr. Jerome Lavelle, pledged Title sponsorship ($5000) for travel for undergraduate engineering students, including Sydney Floryanzia, Alice Di Fazio, Hannah Johnson, Matt Sabo, and Calvin Shanahan. The Poole College of Management at NCSU provided a Bronze Sponsorship ($500) for travel funds for undergraduate student Madisen Andersen. Dr. Frances Ligler and George Ligler, Lampe Distinguished Professor and Dean's Eminent Professor of the Practice of the Department of Biomedical Engineering at UNC-Chapel Hill and NCSU, respectively, have provided Title Sponsorship ($5000) funds to support travel and lodging for Biomedical Engineering Ph.D. graduate students Brendan Turner and Kristina Rivera. The North Carolina State University Office of Undergraduate Research (NCSU OUR) provided an ACC|AC Fellowship grant of $2500 for chemical and electronic material costs of the project. Analog Devices provided technical support for circuit design and provided access to next generation impedance spectroscopy devices. BASi provided guidance for electrochemical MIP characterization. NNF donated fabrication equipment time in their clean room, as well as nano characterization tools.

7. Final Remarks

While performing repeated iterations of MIPs fabrication, we learned that chemistry performed with systematic variation is vital to repeatability and robustness of final biosensor. However, further testing of our system is necessary in order to validate a precise time to result and establish a dose-response curve. Future research will be conducted to optimize monomer formulation for MIP film formation as well as specificity testing

Our team would like to thank the invaluable contributions of UNC REX management, nursing, and medical staff. The clinical insight and willingness to guide us on the realities of vancomycin use and associated cost were critical in shaping the business case. We would also like to thank the SensUs 2018 Jury for their time and attention towards the year-long efforts of the students. Finally, the team would like to additionally thank the SensUs organization for their time and effort into putting together such a wonderful opportunity for students around the world to collaborate for a great cause.

8. References

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9. Appendix

9.1 Business Model Canvas:

<table>
<tr>
<td rowspan="2">PROBLEM
- In the USA, AKI is affecting hospital costs when Vancomycin levels rise too fast in patients' bodies
- Levels fluctuate rapidly with time, making it difficult to track and predict the risk of AKI
- Demand for the hospital lab only allows for weekly blood sampling for patients</td>
<td>SOLUTION
- Create a quicker, bedside accessible means of monitoring that is accurate
- Easy to operate device and interpret results
- Cost effective testing
- Electronic documentation in the patient records</td>
<td rowspan="2">UNIQUE VALUE PROPOSITION
This device will provide an FDA approved, bedside means of monitoring Vancomycin and can be operated in the patient's room, transported from room to room, and can be interpreted immediately by medical personnel, allowing for faster and more informed adjustment of vancomycin dosing.</td>
<td>UNFAIR ADVANTAGE
- Strong biomedical influence in the area (RTP)
- PhD Mentoring
- University Alumni
- Large medical presence in the area
- Sponsorship</td>
<td rowspan="2">CUSTOMER SEGMENTS
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- Medical device distributors</td>
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