Bringing Vancomycin Analytics One Step Closer

Team Results Document

DeTectUs - Technical University of Denmark

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1 Summary for SensUs Website

Sharing the ideal of easing the work of healthcare professionals and improving patient care, the DeTectUs team has developed a small and portable biosensor system which is able to measure the concentration of vancomycin in patient blood samples.

Vancomycin is a drug of extreme relevance since it is the last resort antibiotic used for the treatment of patients with multi-resistant bacterial species. According to DANMAP 2016, approximately 2.000.000 doses of vancomycin were given to patients in 2016 at Danish hospitals. Therefore a system, which is able to measure the concentration of the antibiotic rapidly, easily and precisely is of great relevance to healthcare workers.

The currently presented sensor is based on the simple idea of using a metal substrate to immobilize custom-made entities, which are able to bind vancomycin specifically and induce a measurable signal. During measurements, the sensor chips are placed in a chamber for sample hold and stabilization. The patient sample is then introduced for less than 5 min, followed by a measuring solution, which serves as the measurement liquid. In mere minutes, the output is visualized on a small screen as a matter of vancomycin concentration in the sample. The designed set-up is created for use in hospitals and healthcare facilities and is to be operated by untrained individuals such as nurses or patient caretakers. Changing the way that vancomycin is monitored, analytics can be taken out of the lab and brought one step closer, to the bedside of the patient.

2 Biosensor System and Assay

2.1 Detection Principle

The goal of this year's SensUs competition was to develop a point-of-care (POC) set-up for the measurement of vancomycin concentrations in patient blood samples. Vancomycin is a last resort antibiotic, used for the treatment of patients, terminally infected by multi-resistant bacterial species. The following section will briefly explain the principles of the proposed biosensor for the detection of the antibiotic. However, since the technology is patent pending, technical specifications cannot be disclosed in details.

The current solution involves a custom-made 3-electrode setup fabricated using the cleanroom facilities at DTU Danchip to ensure that the metal surface is as pure as possible. Together with well-designed and synthesized receptors the sensor is able to bind and detect vancomycin selectively and sensitively. Binding entities are chemically linked to the surface of the metal sensors through an immobilization process. Afterwards, surface bound receptors can capture vancomycin molecules in the sample and induce a measurable change in the electrical output. Figure 1 shows a schematics of the sensor surface, immobilized receptors and target binding.

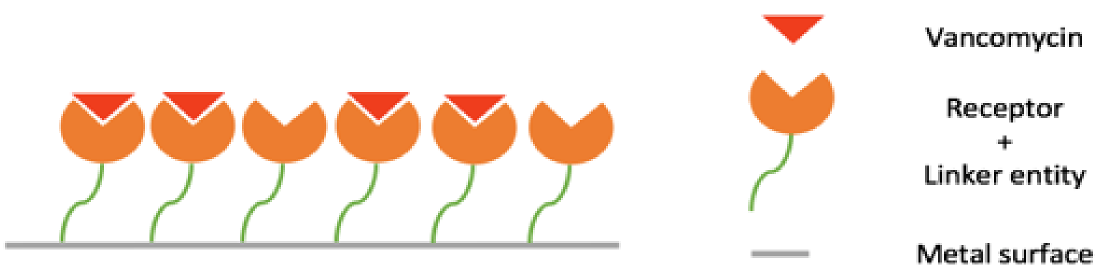

Figure 1: Schematics of metal sensor surface with immobilized receptors and vancomycin bound.

2.2 Set-up and Use

The current sensor setup includes the electrical measuring device, the sensor chips and the measurement chamber.

Chips are prepared with the immobilized receptors in advance and they can be easily and conveniently clipped into the measurement chamber, which fixates the chips and ensures that the sample volume remains in place. A one time use pipette is used to introduce the sample in the chamber, which is incubating on the surface for less than 5 min. After the incubation time, the sample is replaced by a measuring solution, which is used to obtain the signal. Then, the microcontroller of the printed circuit board (PCB) is able to calculate the concentration of vancomycin in the sample and display the result on a small LCD screen. Figure 2 displays the chamber and connector set-up.

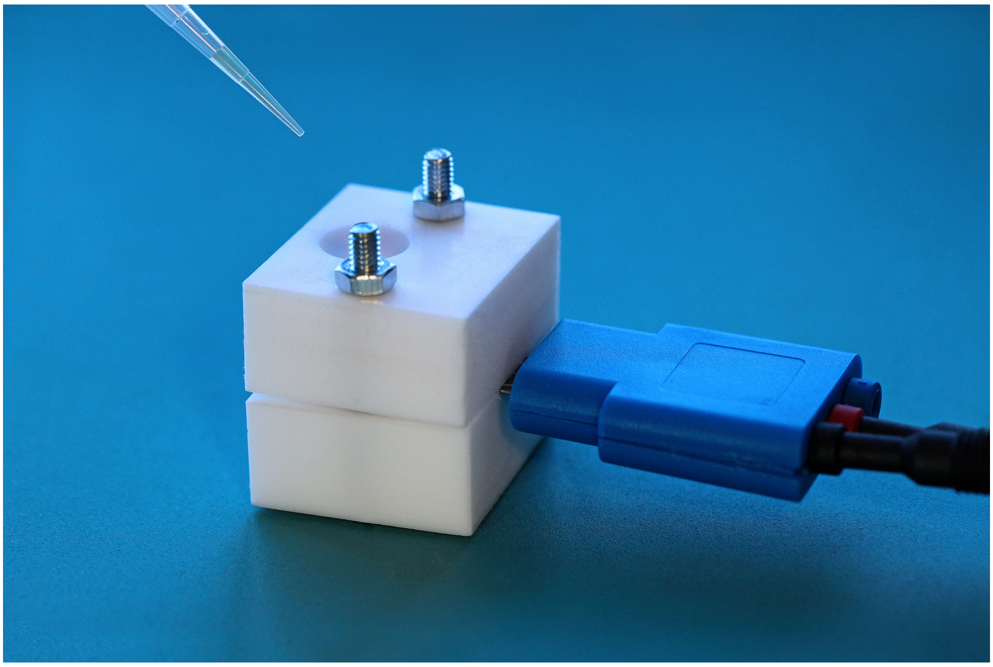

Figure 2: Plasma sample getting introduced into the chamber, which holds the sensor chip. The sensor chip is attached to the connector, which transmits the signal to the PCB.

3 Analytical Performance

In order to evaluate the currently presented set-up, a dose-response curve was established based on the average result of several measurements. Figure 3 shows that an increase of vancomycin concentration in the sample correlates with a higher signal output.

Required sample volume for the incubation is $\sim 10\,\mu$l and the required measuring solution volume for the measurement is $\sim 200\,\mu$l. Neither the sample nor the measuring solution is re-used, however due to the small volumes necessary for a measurement, this is not expected to become an obstacle for future application.

Experiments were carried out in 2-day blocks, where during day 1 the sensor chips were cleaned, characterized and the receptor was immobilized. During day 2, the measurements were performed in the presence and absence of vancomycin in the sample. Chips with the immobilized receptor were stored at room temperature in dark. Vancomycin and plasma were stored frozen, the measuring and receptor solutions were stored in the fridge and buffer solutions were stored at room temperature.

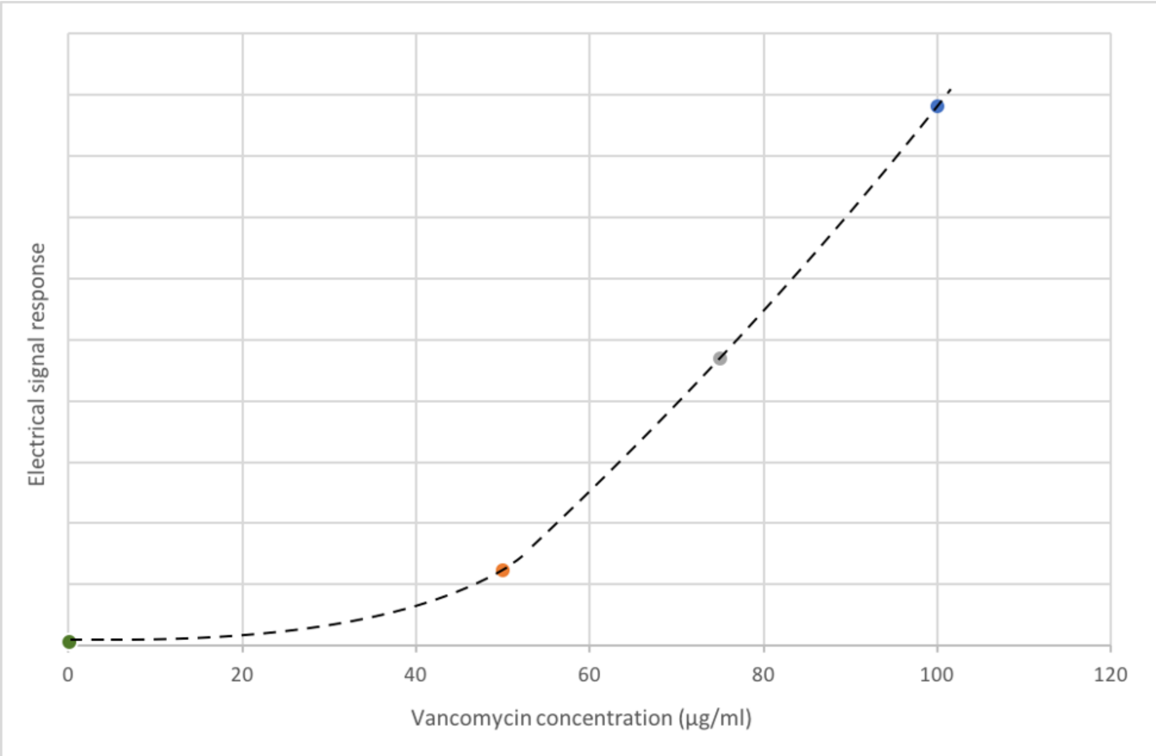

Figure 3: Dose response curve, with measurement points at 0, 50, 75 and 100 μg/ml of vancomycin in the sample.

4 Novelty and Creativity

4.1 Already Available

Methods for the cleaning and characterization of microfabricated sensor chips as well as the immobilization of receptors on metal surfaces are all techniques, which are widely used and freely available. Moreover, the detection principle used in the current set-up is commonly utilized for the quantification of ligand-target binding events on a microscale.

Cleanroom facilities were available for fabrication of the chips, enabling alterations in design and allowing up-scaling for mass production. The masks for fabrication of the 3-electrode setup, as well as the chambers were produced using standard milling processes. CAD programs such as SolidWorks and L-edit were used to design masks. For specific integrated circuit (IC), evaluation boards with inspiration were available. Besides this, licences for different CAD programs (such as Eagle for PCB design) were available for free provided by the university.

4.2 New Development

The main novelty of the currently introduced system lies in the uniquely designed receptor molecule. This entity has been carefully constructed for the current needs of surface immobilization and target binding by selecting each building block individually and getting the receptor synthesized. The work was carried out in cooperation with a private company.

Receptors are attached to custom-made sensor chips, which have also been uniquely designed to fit the current purpose. As mentioned before, during this process state-of-the-art cleanroom facilities at DTU Danchip were used. Metal deposition devices such as sputterers and E-beam evaporation systems were utilized to fabricate metal layers with a high degree of purity. This was to ensure low sheet resistance in the device which would benefit the measurements in contrast to commercially available DropSens electrodes which have also been tested. These electrodes use screen printing as means of fabrication, that results in a lower purity of metal layers.

The electrical measurement system has also been developed and modified for vancomycin specifically and consists of a PCB with the bare minimum requirements for the detection of different vancomycin concentrations, in comparison with the bulky and expensive machines present in common laboratories. The currently designed device is capable of one specific type of measurement, where all ranges and components for both calibration and detection have been chosen specifically for vancomycin. These values are solely based on the measurements done by the surface chemistry/biology team, using the sensor chips which were fabricated by the sensor team. As such, the device is capable of performing precise measurements and comes at a very low cost due to it being fairly minimalistic, taking up an area of only 5x8cm, excluding the user display, which can be replaced by any other user interface (smaller screen, bluetooth, etc.). Therefore, the device is applicable with the current setup only, as well as only for vancomycin.

5 Translational Potential

The following sections are primarily based on an interview with Ida Gjørup, senior doctor at the department of infectious diseases in the Copenhagen University Hospital.

5.1 Stakeholder Desirability

Vancomycin is used for the treatment of serious, life-threatening infections by Gram positive bacteria unresponsive to other antibiotics. Even though vancomycin only counts for a significantly small amount of the total antimicrobial agents administered to patients, in 2016 approximately 2.000.000 doses of vancomycin were given in Danish hospitals (DANMAP 2016). One of the stakeholders with high power and interest, the doctors from Danish hospitals were interviewed in the spring 2018. From the interview with Ida Gjørup, it was clear that the there is a need for a quick yet highly precise test. Presently, the doctors can order a laboratory technician to perform the vancomycin test but each department only has specific time slots where tests can be taken, due to the fact that other departments order tests as well. Because of the processing time for the test, the doctors who order the vancomycin test, will not receive the results before their shift is over. As an effect of this, the doctor at the next shift will receive the results and has to get to the patient and find out what to do and exactly how to continue the treatment. With a fast, easy and accurate method and tool to test vancomycin at each department, the nurses could perform the test and the same doctor who ordered the test could get the results within his/her shift and thus ensure the patient's safety and correct treatment at the right time.

The main stakeholders are listed in Figure 4 below according to their level of power and interest in the project. A brief explanation of the coordinate system can be seen in Figure 5 as well.

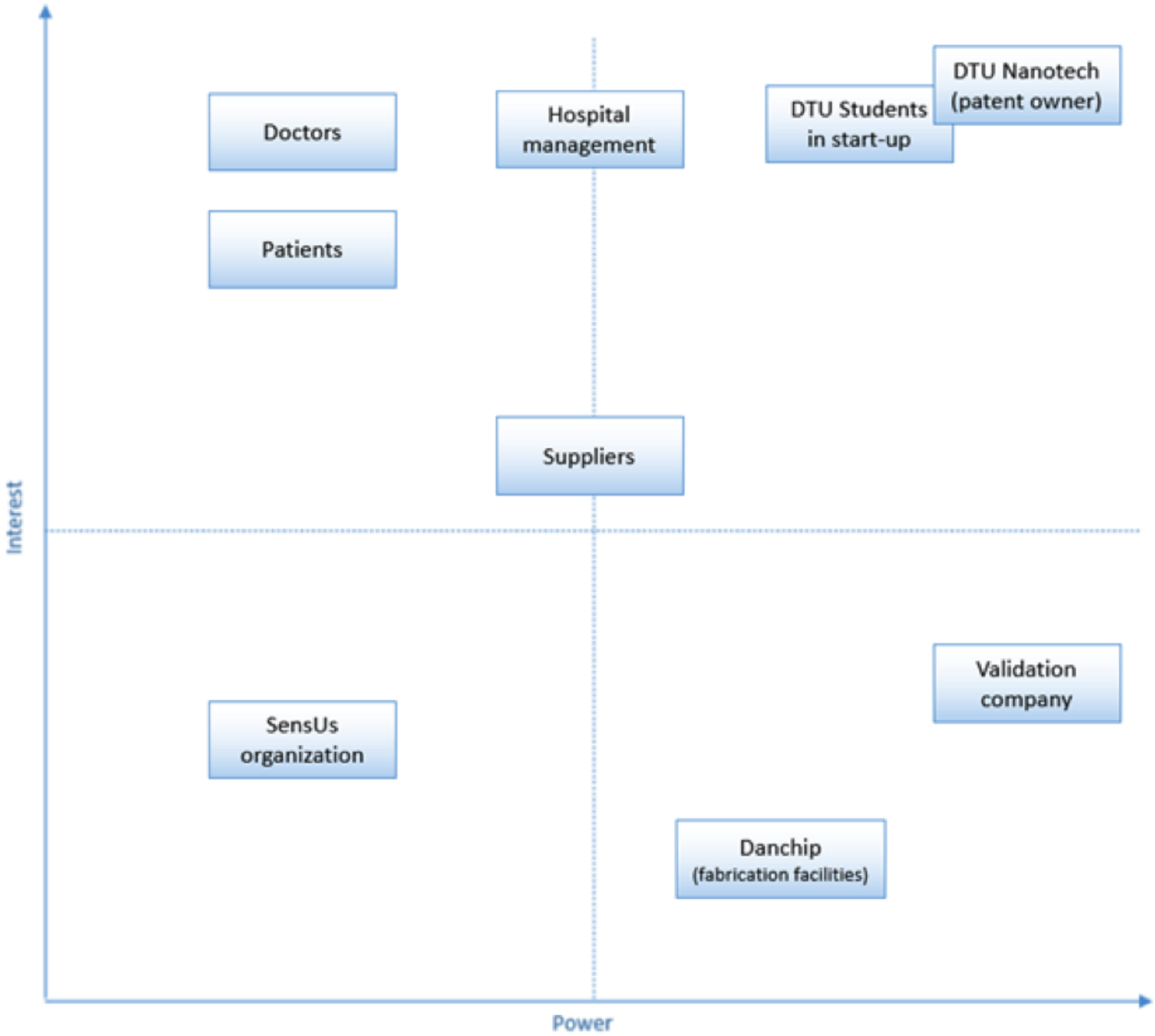

Figure 4: Stakeholder map divided according to power and interest.

The highest ranking groups are the DTU Nanotech institute as well as the DTU students behind the invention, as they are the patent and project owner, respectively. In almost equally high interest lies the patients, doctors (and other hospital staff involved in the treatment of patients) as well as the hospital management – meaning the customers and users. The latter has a little more influence than the others, as they are the ones making the final call whether or not the hospital purchases the actual sensor, whereas the doctors and patients are primarily concerned about getting the right treatment faster. The suppliers are placed in the middle as they have a certain influence as long as they are needed for the purchase of components. It is important to note that they can also be of risk, if their power is not properly managed.

The validation company has an even higher influence, as they can close the whole production site if they find incompatibilities.

Lastly, the stakeholders can move both influence and interest depending on the state of the situation. Figure 5 shows the power/interest matrix of stakeholders. For now, the project is not of relevance to SensUs as an organization or to Danchip/DTU Nanotech, as providers of the experimental space. However with the change of ownership rules, regulations or partnership, the situation could easily look different in the future. It is expected for the future that all parties of relevance such as DTU Nanotech and the founders of the start-up, will share the same level of power and interest in an increasingly professionalized setting.

Figure 5: Explanation of the Power/Interest matrix for the stakeholders.

Closely linked to a customer profile and pains & gains, stands the value proposition. It's a strategic decision making tool for understanding the customer and designing something of actual value. Figure 6 shows the left side of the proposition, called a value map, displaying the gain creators and pain relievers of the product. The value proposition is simply defined as a tool to detect the level of vancomycin in blood plasma fast, easily and accurately to accommodate the busy schedules of doctors and nurses in hospitals.

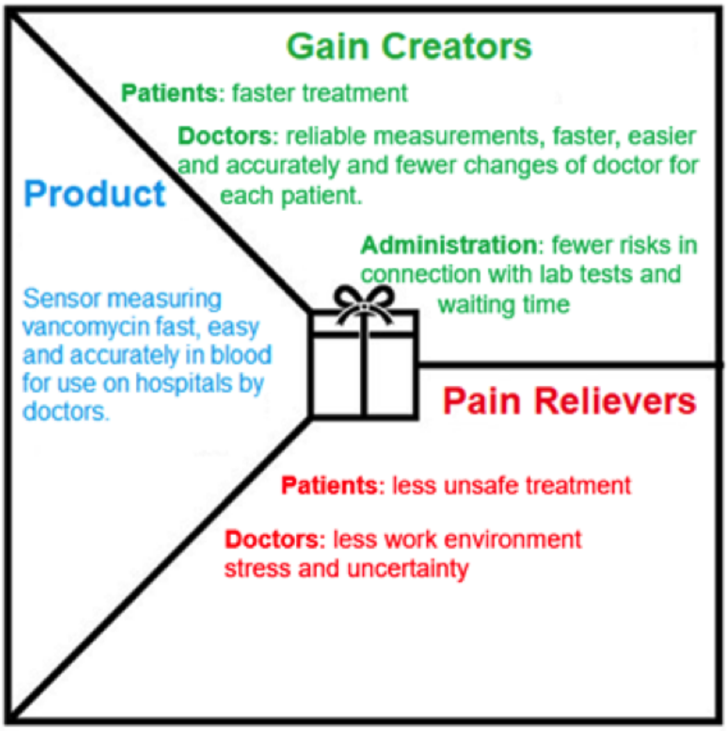

Figure 6: The left side of the value proposition map, showing the value map itself and the designed choices for pain relief and gain creation for the costumer segment.

5.2 Technical Feasibility

The presented sensor set-up is envisioned to be used in a broader spectrum than for the measurement of vancomycin. As the system is optimized for blood plasma and can be further developed for whole blood analytics, essentially any kind of biomarker present in blood could be of relevance. This opens up a huge market potential where both healthcare facilities and individual patient groups, may be the target customers. Other measurement systems for vancomycin analytics are currently available on the market such as the Beckman Coulter Emit 2000 Vancomycin assay as well as various measurement kits developed by Roche. These systems are based on the principles of an immunoassay and they use specific antibodies to capture and detect vancomycin (Beckman Coulter, 2010 & Courtney H. Lias, 2016) . Even though these systems have a low detection limit and excellent sensitivity, there is a lower limit of market price that they can aim for without risking the loss of revenues, since antibodies are high cost biological components. Both the Beckman Coulter and the Roche system uses blood plasma or serum as the sample and not whole blood. If further development of the current system follows expectations, then whole blood blood may be used for vancomycin analytics, which can serve as a point to overcome other competitors. Since a patent has been filed by the Technical University of Denmark for the currently presented invention, the first step has essentially be taken in order to form a company and develop the product further for commercialisation. As a future aspect, the measurement set up and the sensor chips may be provided together with single-use pipettes in the form of a kit with potential filter units to aid the use of whole blood as an initial sample for analytics. To keep the device at a low cost, all fabrication, surface chemistry and calibration steps are planned to be carried out in house, with the help of externally hired technical consultants who can periodically help with the preliminary development phases. A further aim of the current system is to help hospitals reduce their costs by reducing the amount of time patients need to be hospitalized. Based on the talk with Ida Gjørup, it costs approximately 10 000 DKK or over 1000 EUR for one patient to be hospitalized for one day. Therefore, if the current device can aid the recovery of patients and their faster return home, that will result in huge economical advantages to hospitals.

5.3 Business Viability

Figure 7 shows the business model canvas for a potential start-up with the vancomycin sensor. An important thing to notice is that the customer is not the same as the user. The paying part is the hospital management deciding to use the sensor, whereas the user is the doctors and nurses treating the patients and are thus direct and indirect users. There will be in the beginning a very direct contact to the buyers to ensure optimal service and product quality. When this is running smoothly, a more indirect communication form is more optimal for both parts.

The primary revenue streams will come from direct sales in a model similar to perpetual licensing maintenance. Attention should therefore be directed towards the sales period after the hospitals get to try out the sensor before they purchase it, in order to - in fact - have a revenue stream.

Like mentioned in the stakeholder analysis, the key partners are industry collaborator for fabrication of chips, suppliers and of course an external party to validate the quality of the sensor. A large part of the costs structure goes towards these key partners as well.

Note that service and calibration teams are also mentioned at both key activities, under channels and costs as well as revenue stream. It is believed that such a team is necessary to keep up the high quality of both the product and the service to the customers.

It is of relevance to consider the argument that the market is fairly small as the sensor is currently very specific. However, according to Mrs. Gjørup from Copenhagen University Hospital, two patients infected by resistant bacterial species are treated with vancomycin every month at their hospital, assuming other hospitals have roughly the same or even less, its still 100 people a month, thus creating a need for a treatment that is faster and more convenient than current methods.

5.4 Development Timeline

In order to evaluate the future perspectives of the sensor system, it is important to establish some main milestones of development in a potential start-up until the point that the sensor system can enter the Danish market.
1. By the end of 2018, fundations of the strat-up are aimed to be settled and the preliminary development phase is aimed to be over. The sensor should be able to measure vancomycin levels in blood plasma, sensitively and selectively at a lower limit of detection that enables the set-up to compete on the market. Parameters will be determined, based on a thorough study of the competition and customer needs. Interference levels from other antibiotics which may be present in blood plasma will be evaluated and accounted for.
2. By the end of 2019, the system is aimed to be integrated in an easy to handle, portable set-up, which is ready to be operated by non-trained individuals. Studies with blind samples and result comparison to currently used machinery will be of main focus. In order to ensure easy handling, system kit prototypes will be handed to hospitals and healthcare institutions for no charge and their feedback will be closely monitored for quality assurance and development purposes.
3. By the end of 2020, the start-up should be ready to launch the product on the Danish market. Focus will be taken to advertising and commercializing as well as the investigation of additional application possibilities for the device.
If everything goes as described above, further milestones for the upcoming years will include the development of additional detection systems as well as expansion to foreign markets.

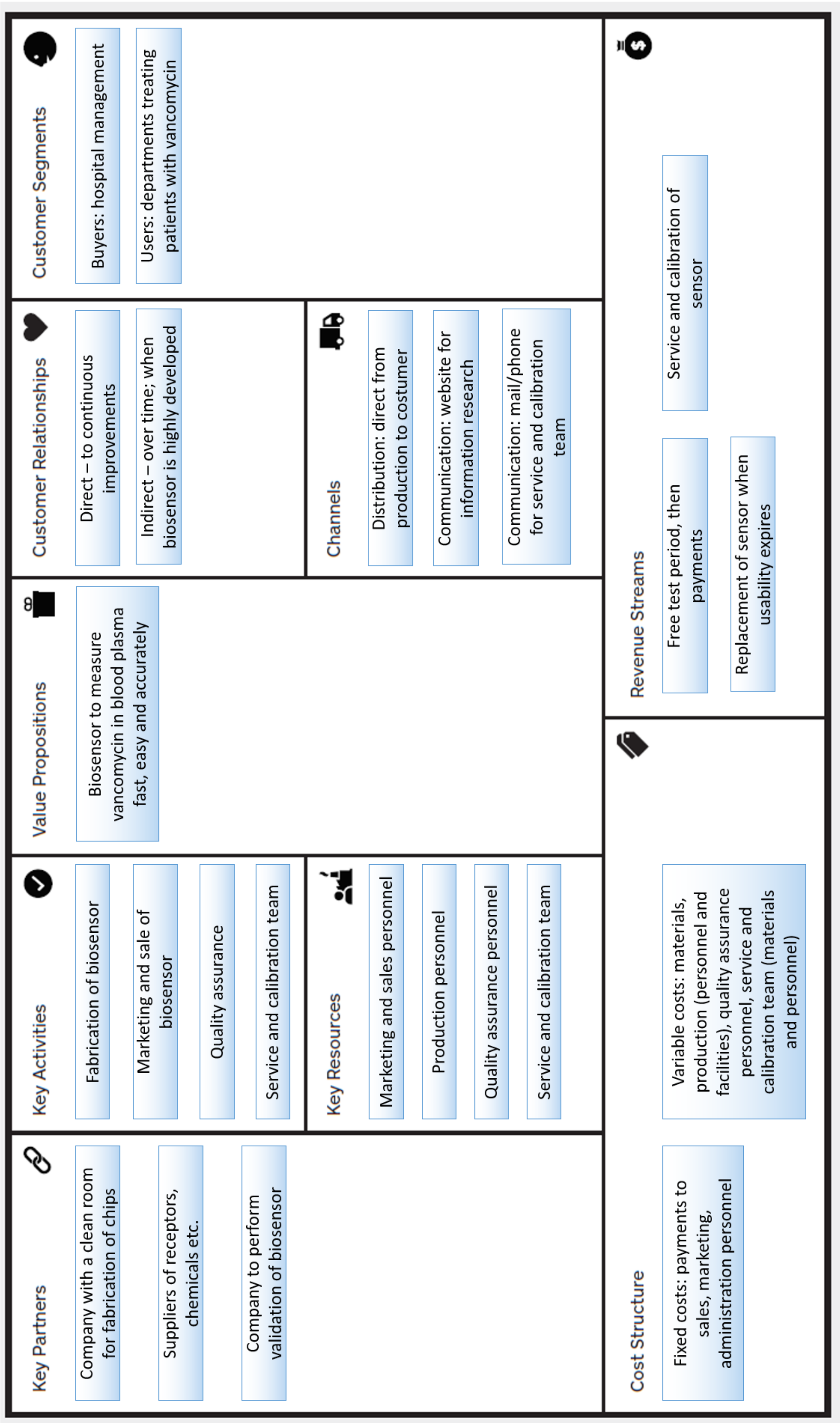

Figure 7: Business model canvas

6 Team and Support

6.1 Contributions of Team Members

Subteam	Responsibilities	Team Members
Surface chemistry/biology	Cleaning and characterisation of metal sensors. Immobilization of receptors and measurements.	Zsófia, Christopher
Sensor fabrication	Fabrication of sensors, including passivation and metal deposition. Fabrication of chambers for electrodes.	Kitty, Magnus, Nicolai
Electrical setup	PCB design, microcontroller coding, board soldering, component ordering, and calibration.	Berk, Kristian
Translational potential	Development of business plans, models and potential of future industry collaboration possibilities.	Amalie, Mads
Outreach	Outreach on different social media platforms making people aware of the work carried out by the DeTectUs team.	Stine, Kitty

6.2 People Who Have Given Support

Natalie Kostesha, senior researcher at DTU Nanotech:
Natalie has been supervising the whole team, helping with both the layout of the fabricated chips and the experimental work. She has been especially supportive to the biology team in the lab-work and procedures. Throughout the project she has helped solved problems and inspired idea generation for the team.

Maria Dimaki, senior researcher at DTU Nanotech:
Maria has been a great help in supervising/overseeing the cleanroom work done by the sensor fabrication team, and has always been available for consultancy on the fabrication of the device.

Erling Nielsen, system architect at PlastiSens:
Erling pointed the electrical team in the right direction, predicting potential problems the team might run into and giving a basic introduction to what the team is dealing with. He also suggested a few key components, which made the electrical aspect of the project function.

Ida Gjørup, senior doctor at the Department of Infectious Diseases at the Copenhagen University Hospital:
Ida helped the team through an interview, where she explained the current treatment methods for patients infected by resistant bacterial species. She detailed the use of vancomycin as well as a potential need for a sensor set-up, which can ease rapid analysis of antibiotic levels in patient blood samples.

6.3 Sponsors

Xellia:
Did a presentation on vancomycin to give the team a better understanding of the antibiotic, its production, uses and characteristics.

PlastiSens:
Helped out the team with knowledge to produce the electrical setup.

7 Final Remarks

The team would like to thank the whole NaBiS group of DTU-Nanotech for their help and support with the planning, the practical work as well as problem solving, whenever the team encountered challenging situations.

Furthermore, the team would like to thank the business developer team of DTU-Nanotech for filing a patent application for the invention and helping with the disclosure of information.

The team is very content that the university believes in our idea and the team would like to proceed with the establishment of a start-up company, where the development would be further pursued in order to meet standards of the industry.

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