SensUs 2018

Team Results Document

VancoSense

KU LEUVEN

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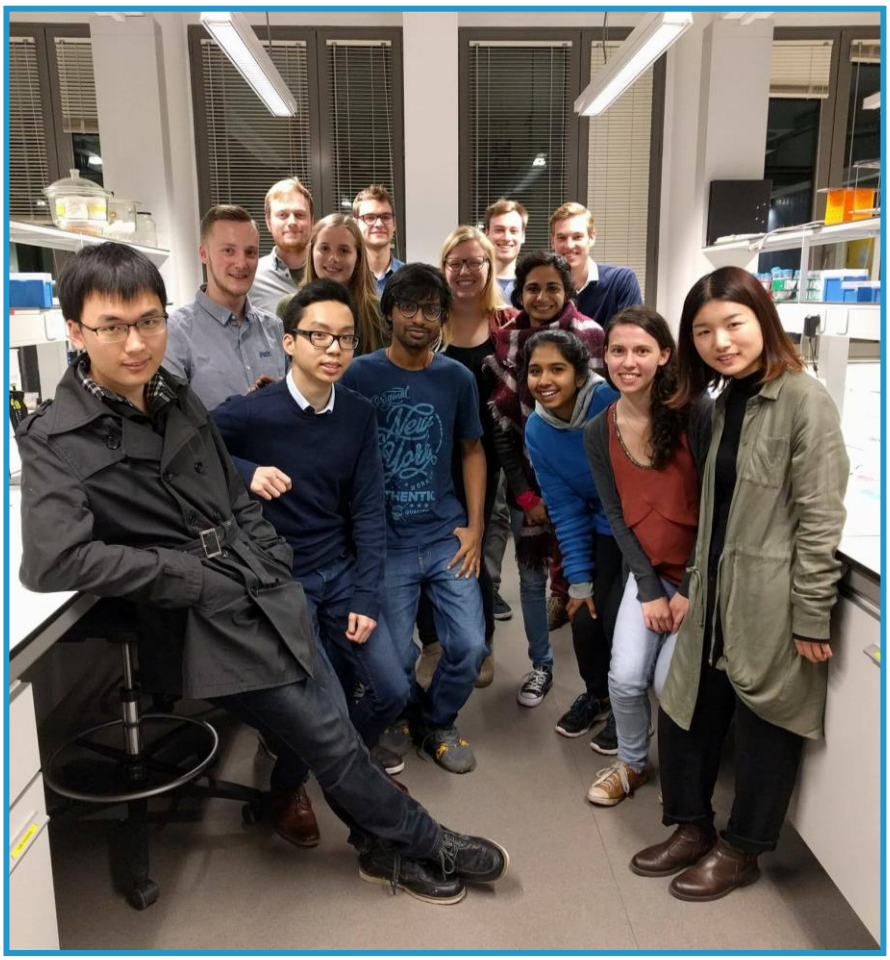

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1. Summary

Travelling all the way from Belgium, team VancoSense is presenting their electrochemistry-based sensor in Eindhoven. The team, consisting of 14 KU Leuven students from all over the world, worked many long days and nights to produce a working prototype. Their sensor uses a carbon nanotube electrode chip, functionalised with a peptide containing a terminal D-Alanine-D-Alanine motif. This D-Ala-D-Ala motif, which is targeted by the antibiotic and enables it to impair the bacterial cell wall synthesis, is employed by the sensor to “capture” vancomycin from the complex plasma sample. The result is the VancoSense, a desktop-sized biosensor, capable of detecting vancomycin concentrations in plasma in under 5 minutes. This sensor could vastly reduce the cost of vancomycin treatment by allowing for a tighter monitoring of the patient’s blood vancomycin levels and therefore reducing the risk of catastrophic side effects. Furthermore, the VancoSense can easily be adapted for electrochemical detection of other molecules with a narrow therapeutic index, which can moreover be detected at the same time.

2. Biosensor System and Assay

Introduction – Due to its low mass and molecular volume, applying established biosensing methods for the detection of vancomycin is not straightforward. However, the presence of phenols in vancomycin's molecular structure provides an often-overlooked alternative: electrochemistry. When an electric potential is applied to a vancomycin sample, these phenols are oxidised, generating an electrical current proportional to the vancomycin concentration. This idea lead to the development of the VancoSense, a desktop-sized biosensor capable of electrochemical quantification of vancomycin on a small, disposable electrode cartridge.

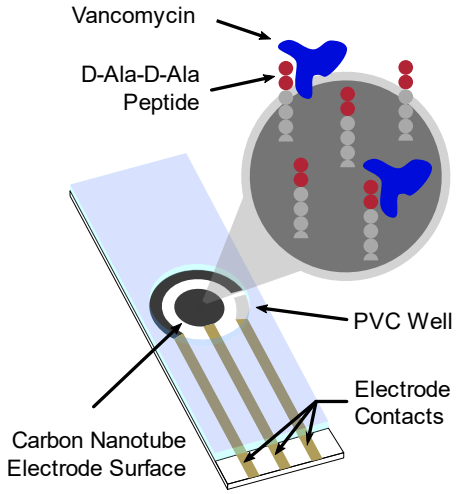

Figure 1. Electrode chip showing the surface, functionalised with D-Ala-D-Ala peptide and the PVC well

Screen-Printed Electrodes & Assay – The VancoSense Device employs a disposable, ceramic screen-printed version of the classic three-electrode setup (Figure 1). The working electrode surface consists of multi-walled carboxylic acid carbon nanotubes, which greatly improve the active electrode surface and provide reactive sites for covalent chemistry. A synthetic peptide, containing two terminal D-Alanine residues, was covalently attached to the electrode's carbon nanotube surface using EDC/NHS cross-linking chemistry (Figure 2). This synthetic D-Ala-D-Ala peptide mimics vancomycin's natural binding partner and acts as the biosensor's biological recognition molecule, binding vancomycin molecules and keeping them in closer proximity to the electrode surface.

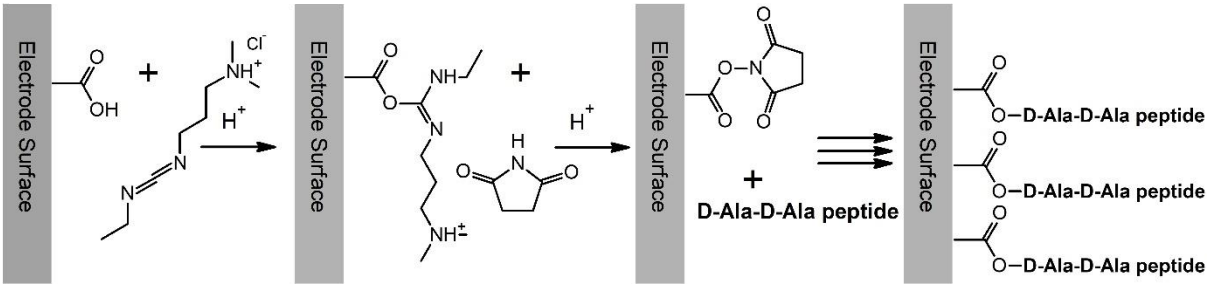

Figure 1. EDC/NHS linking of D-Ala-D-Ala peptide to carboxyl groups on electrode surface

In blood plasma, vancomycin is bound to the hydrophobic pockets of Human Serum Albumin (HSA), lowering the amount of free vancomycin in the sample[1, 2]. The bound vancomycin can be released from HSA and other proteins by denaturation. Therefore, the first step of sample preparation is to mix an equal volume of plasma and methanol and to vortex for 1 minute. The sample is then further diluted with 3 volumes of Phosphate Buffered Saline (PBS), providing the electrolytes, required for electrochemical measurements, and reaching a final plasma dilution of 1/5. A 30 µL droplet of the resulting diluted plasma mixture is then pipetted into a PVC well on the electrode chip, ensuring a reproducible surface coverage and reducing the total sample volume needed.

Since electrochemical methods are highly temperature dependent, a heating chamber was built to surround the electrode and keep it at a constant temperature. The electrode cartridge is inserted into this chamber by means of a reliable and user-friendly sliding mechanism. The slider assures a reproducible contact between electrode cartridge and receiver. After an incubation time of 3 minutes at a temperature of 37 °C in the heating chamber, the VancoSense potentiostat module applies a specific voltage and simultaneously

measures the resulting current. From this current in time reading, the plasma concentration can be calculated. The specific oxidation potential used during amperometry is determined beforehand in the lab. See section 3. Analytical Performance for graphs and details on voltammetry and amperometry.

Signal processing and case – A Raspberry Pi miniature computer and surrounding electronic circuits read the analog potentiostat output and turn it into a comprehensive result, which is displayed on a touchscreen. The touchscreen allows for user-interactions such as stopping and starting measurements and exporting data. The Raspberry Pi also controls the temperature of the heating chamber through a closed control loop between the heating element and a temperature sensor. An integrated battery unit improves mobility of the sensor, making measurements at the patient's bedside possible. All the hardware is enclosed in a plexiglass case, with a total mass of 1.2 kilograms.

3. Analytical Performance

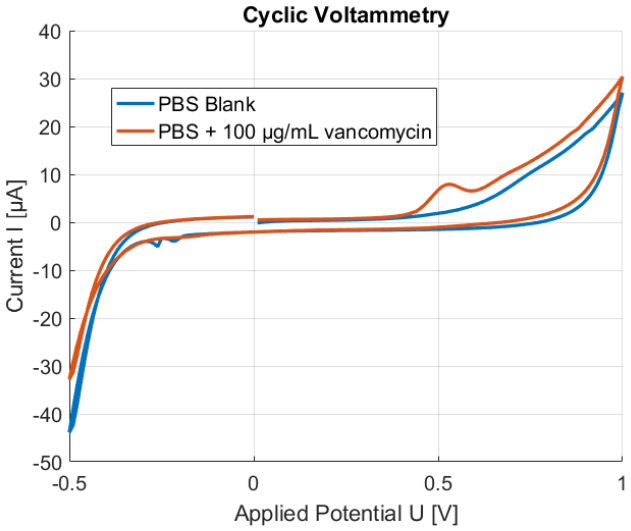

Figure 2. Cyclic voltammetry of vancomycin-spiked PBS. This experiment was repeated 2 more times (not shown).

Determining a calibration curve in PBS was carried out as follows. First, vancomycin's specific oxidation potential was determined using cyclic voltammetry.

30 µL of vancomycin-spiked PBS is loaded onto an electrode chip. Then, a voltage sweep was applied, and the resulting was current measured (voltammetry). At vancomycin's specific oxidation potential, the plasma-spiked PBS sample generates a higher current than the blank PBS sample (figure 3), visible as an oxidation "spike" on the voltammetry curve. The potential where the signal to noise ratio is highest was taken as the specific oxidation potential of vancomycin (0.52 V in PBS). An analogous approach can be used to determine the oxidation potential of vancomycin in plasma.

Subsequently, amperometry was used to determine the dose/response behaviour of the sensor. Different samples of different vancomycin

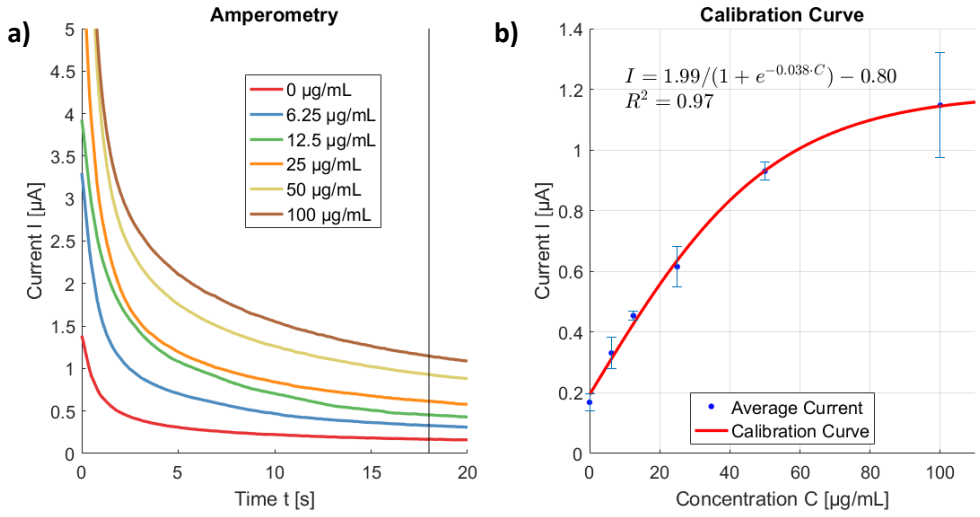

Figure 3. (a) Amperometry of different vancomycin concentrations in PBS, each curve is the average of 3 repetitions. (b) Calibration curve of amperometric current at 18 s versus vancomycin concentration

concentrations were loaded onto separate electrodes. Then, vancomycin's specific oxidation potential was applied, and the resulting current was measured in time (amperometry). A non-linear equation was fitted to the current at 18 seconds versus time, resulting in a fitted model with an R^2 value of 0.97.

With a calculated limit of detection of 1.76 µg/mL, the VancoSense is capable of quantifying vancomycin in PBS within the therapeutic range of 0 to 100 µg/mL. The PBS sample volume used is 30 µL. Time to result is 18 seconds plus sample handling, estimated at 3 minutes. Measurements in plasma have proven to be more troublesome – Human Serum Albumin (HSA) present in the plasma binds vancomycin and thus reduces the free vancomycin available for the sensor[1, 2]. This problem was solved by diluting the plasma with methanol which denatures the HSA. Furthermore, pH variations in the sample after dilution has made producing a vancomycin calibration curve in plasma impossible thus far. For now, the sensor is capable of differentiating plasma samples of 500 µg/mL (= 100 µg/mL after 5x dilution) vancomycin from blank samples. The team will be working around the clock to solve this problem and quantify vancomycin in plasma before the competition event.

4. Novelty and Creativity

Electrochemistry is one of the oldest sensing methods for use in biological samples, starting with Clark's oxygen electrode in the 1950s. The potentiostat module that is incorporated into the sensor is provided by Metrohm, together with the unfunctionalized, screen printed electrodes.

Electrochemical detection of vancomycin using functionalised electrodes has not been published before. The VancoSense biosensor directly detects vancomycin without using an intermediary assay step, leading to a short assay time and simple sample preparation. No pre-purification of the serum (such as chromatography) is necessary. Furthermore, only a limited number of publications report the use of an antibiotic's natural binding partner as a specific capture element.

The protocol for functionalising the carbon electrode surface with the D-Ala-D-Ala peptide was developed in-house. Furthermore, the team wrote a custom-made signal processing program on the Raspberry Pi and developed their own graphical user interface on the touchscreen. In addition, the temperature-controlled heating chamber with slider mechanism, the PVC well on the electrode surface and the complete sensor case were designed and manufactured by the VancoSense team.

5. Translation Potential

Stakeholder desirability - Vancomycin is a low therapeutic index drug of which the concentration in the patient's body must be maintained between 15-20 mg/kg for the drug to optimally act on bacterial infections[3]. Too low concentrations (below 10 mg/kg) have shown to cause vancomycin resistance due to low dose exposure whereas too high concentrations (above 70 mg/kg) have shown to cause irreversible ototoxicity and nephrotoxicity. To prevent these disastrous consequences of inaccurate vancomycin dosing, periodic monitoring of blood vancomycin levels is essential. However, according to dr. Bhide from Sasoon hospital (India), in most parts of the world vancomycin monitoring is not done, primarily due to logistic reasons and secondly due to the time taken for the tests. Even in Europe, vancomycin is only measured once or twice a day according to statements from a doctor in Parma (Italy) and prof. dr. Alexander Wilmer from Leuven (Belgium). In smaller hospitals, such as in Overpelt (Belgium), vancomycin is even measured only once every 2 days. This is insufficient and could pose a huge threat to public health, since vancomycin is the last resort in treating many infections and resistance to this drug can take us back to the pre-antibiotic era where patients might die of throat infections.

In the Benelux, about 11 000 patients are administered with vancomycin per year. With the current Belgian population being 11.4 million (40 % of Benelux), it is estimated that around 4400 patients in Belgium are treated with vancomycin on a yearly basis. About 10 % of these patients suffer from kidney failure as a side-effect[4]. In Belgium, the government spends about 37 000 euros in treating the case of a single kidney failure[5], thus amounting to a total expenditure of ± 16 million euros every year (Table 2 in Appendix). Moreover, due to the complications by the inappropriate dosage of vancomycin, the rate of readmission of patients increases, which lowers the hospital rating and causes the government and insurance companies to lose large sums of money for the further treatments. Furthermore, in case bacterial resistance against vancomycin would develop, the exorbitant amount of money which will have to be spent on synthesizing a new antibiotic is beyond comprehension.

With current techniques, testing vancomycin dosage takes a long time. After taking a blood sample, it takes 4 hours to 1 day to get the results, depending on the specific hospital. In contrary, our device VancoSense, facilitates vancomycin measurement by reducing the time-to-result to less than 5 minutes, allowing multiple test runs per day. VancoSense is a small, point of care medical device which can be used at the ICU, near the patient's bed. With a user-friendly slider mechanism and a touch-screen, it does not require trained personnel to operate. VancoSense will eliminate long waiting times, caused by samples being stalled in clinical laboratories, allowing doctors to have a better control over fast recovery of the patients, reduce the rate of readmission in hospitals, and save a lot of money from government and insurance companies. With our device, side-effects such as renal failure can be reduced to at least 50 %, saving the government about 8 million euros per year.

Technological feasibility - For mass production of VancoSense, we will outsource the fabrication of the device to the design and manufacturing company Comate. Furthermore, we have a collaboration with Metrohm, who will provide us with the potentiostat modules and electrodes. The functionalization of the electrodes and the further R&D will be done internally. After production and clinical validation of VancoSense, our efforts will be focused on miniaturizing VancoSense to a hand-held device, the "VancoPager". Apart from detecting vancomycin, this device will be further developed in parallel with new cartridges, capable of detecting other therapeutic drugs. Dr. Bhide (India) taught us about other low therapeutic drugs besides vancomycin like digoxin, aminoglycosides, phenytoin, phenobarbital, carbamazepine, and valproate, which require close follow-up of dosage as well[6]. Furthermore, from the doctor's perspective, multiplex detection to obtain the concentration of multiple drugs in a single blood test will be very useful since often a cocktail of various drugs are administered to the patient. This second-generation device will be referred to as "VancoPlexer".

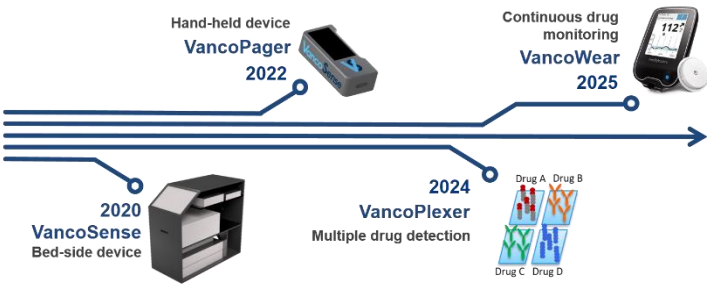

Figure 4: Technological time-line of future devices.

Our third-generation device will be the Continuous Vancomycin Monitor (CVM) system, which will perform real-time measurements of the vancomycin concentration. Since the concentration of vancomycin in interstitial fluid (ISF) and blood is comparable[7], this device, called "VancoWear", will be minimally invasive. VancoWear will have a transmitter and external pocket device to which the readings can be sent wirelessly. The data can be sent to a doctor, who can remotely monitor and act upon the measured vancomycin concentration[8]. As the specialists vary dosage, based on data received from the sensor and the patient's medical history, we can collect massive amounts of big data about this decision. Eventually, with a large enough dataset, we will be able to use risk factors and probability functions to predict the appropriate dosage of vancomycin. This algorithmic decision making,

guided by specialists, will improve drastically over time and eventually will reach a point where it becomes as accurate and efficient as consulting specialists[9,10,11]. This way, VancoWear can be connected to the vancomycin IV drip, programming it such that the drip rate is automatically altered according to the reading and hence approaching the dream of having an ideal vancomycin concentration in the patient's body for a long period.

Business viability – In recent years, a trend in the European healthcare system can be noticed, where 40 % of the medical devices are leased rather than bought[12,13]. This is because the hospitals, as an enterprise, want to reduce their capital expenditure so that they can have cash on the balance sheet to invest in other services that provide better care for their patients. Therefore, we will lease our device to the hospitals via a 5-year contract and charge a fixed price per cartridge. This way, we become an operating expense, thereby increasing the probability of the hospitals choosing for our device. Another benefit of this model is that we earn consistent recurring revenues and can also charge the hospitals a maintenance fee. Whenever we release newer versions of the devices (e.g. VancoPager), the hospitals will not have to think about new investments to replace their current devices. We will charge an adjusted lease fee for the new devices and take back our older ones. This way, we facilitate the sales of new devices to established customers and sell the older ones to customers that do not want to pay a premium for the latest device.

The device will be leased at a fee of 13 000 euros per year, while the selling price per cartridge will be 10 euros. With an estimated amount of 1 200 cartridges per device, the total revenue is calculated at 25 000 euros per year. To treat 11 000 patients in the Benelux per year, and assuming 30 patients per device[14], we will be able to lease 366 devices per year. With a cost price of 975 euros per device, 1.5 euro/cartridge, and an initial investment of 20 million euros, covering clinical trials and operational expenses, we will reach a breakeven point at after 2.5 years[15] (Table 1-3, Appendix). In order to protect and ensure our market share, part of the investment will be used to patent our unique combination of using the D-Ala-D-Ala peptide as biological recognition element and the amperometric read-out technique. In the future also other technological patents will be foreseen for the design of both the cartridge and read-out device.

Our immediate target market is the Benelux, with a count of 11 000 patients per year, implying a market of at least 350 devices. Referring to the interview by SensUs with Jaap van Dissel, Director of the Centre for Infectious Disease Control of the Dutch National Institute for Public Health and the Environment (RIVM), he states that "Countries in Southern Europe count up to 3 to 4 times more daily doses of antibiotics per 1 000 inhabitants. This higher usage of antibiotics results in a higher prevalence of antibiotic resistance in health care facilities." Therefore, we want to expand our market beyond the Benelux area to other parts of Europe after 2 years, where our device will be of even greater use. With a rate of 0.4 out of every 1 000 people taking vancomycin, about 300 000 patients will use vancomycin in a population of 741 million in Europe, thus leading to a market of 10 000 devices in entire Europe.

The devices of VancoSense will optimize therapeutic drug monitoring, leading to a breakthrough in health care in the benefit of the patient. We are strongly convinced that our innovative product and hospital-friendly business model will give us a clear advantage over our competitors and will ensure adoption amongst hospitals and insurance providers in Europe and other markets.

6. Team and Support

6.1. Contributions of the team members

Using the *Divide and Conquer* strategy, we divided our team of 14 students in 3 smaller groups. The bioassay team, consisting of Bart T., Bent, Jolien, Kowsar, Mariska, Tijs and Yuting, focused on developing a working electrochemical assay. Secondly, Bart vdZ., Florian and Indrayani, the sensor technology team, made sure we

were able to perform our measurements properly by combining all the hardware into a working device'. Lastly, the signal processing team, consisting of Joris, Junbo and Lore, programmed the Raspberry Pi and the touchscreen and assembled all accompanying electronics. To bring our task to a successful end, all teams worked together closely. The bioassay and sensor technology team, for example, worked together in developing a user-friendly case for the bioassay; while the signal processing and sensor technology team worked together on the heat pad and potentiostat integration.

Next to this Indrayani, Bart vdZ, Kowsar and Lore created the business plan, while Florian maintained contact with our sponsors. Finally, our website and Facebook page were managed by Bent.

6.2. Supporting Researchers

VancoSense would never have been able to obtain these results without the support of Jolien Breukers, Dries Vloemans, Karen Ven, dr. Karen Leirs, dr. Devin Daems, dr. Filip Delport, dr. Jaroslav Belotserkovsky and prof. dr. Jeroen Lammertyn.

We are also grateful to Jonathan Smet from Comate and Jeroen Lybaert from Metrohm, for all the help and advice provided during the development of our biosensor. We would also like to thank Mayank Kale (CEO at Invoq Health) for sharing his expertise of business model development in Healthcare sector. Furthermore, dr. Devavrat Bhide, prof. dr. Alexander Wilmer and Stefanie Smet for providing valuable insight into possible benefits of introducing VancoSense in hospitals.

6.3. Sponsors

VancoSense considers it an honor to have received the support of Metrohm, Comate, MyCartis, Lcie and KU Leuven Research and Development as our main sponsors. We thank them for their financial support and advice. Furthermore, we would also like to thank KU Leuven and MeBioS for the support and facilities.

7. Final Remarks

As a final remark, we would like to thank the SensUs organisation and the TU/e for giving all teams this incredible opportunity. In the past 3 years, SensUs has booked great success in drawing attention to under-appreciated biomarkers. The event week itself provides an awesome networking opportunity in an international context, while the prestige of the competition grows steadily. The KU Leuven hopes to contribute with new teams in future editions and to support the TU/e in promoting biosensor research!

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Appendix

Table 1: Overview of costs of goods, leasing price of the device and selling price of the device

Costs of goods (€)			
Device		**Electrodes**	
Potentiostat	500	Bare electrode	0,75
Casing	75	Fuctionalization reagents	0,75
Hardware and software	400		
Total	975	**Total**	1,5
Leasing price per year (€)	13000	**Selling price per cartridge (€)**	10

Table 2: Overview of sales estimations

Description of estimations based on literature	***Number***
Number of patients in Benelux per year	11000
Number of patients in Belgium per year	4400
Percentage renal failure due to vancomycin	10
Number of kidney failure patients due to vancomycin in Belgium	440
Costs for Belgian government to treat 1 patient with kidney failure	37000
Costs for Belgian government to treat patients with kidney failure due to vancomycin	16280000
Patients per device	30
Tests per day	4
Average number of days of vancomycin treatment	10
Number of cartridges per patient	40
Number of cartridges sold per year per device	**1200**
Number of devices leased (Belgium)	**146,6667**
Number of cartridges sold per year (Belgium)	**176000**
Number of devices leased (Benelux)	**366,6667**
Number of cartridges sold per year (Benelux)	**440000**

Table 3: Overview of break even analysis

Description	Price (€)
Fixed costs	
Initial investment (R&D costs, Staff, clinical trials, ...)	20000000
Variable costs	
Costs per device	975
Costs per cartridge	1,5
Revenue	
Per device per year	13000
Per cartridge	10
Gross margin	
Per device	12025
Per electrode	8,5
Break even calculations	**Number**
Number of units to break even (= 1 year device + cartridges)	899,8875
Estimated number of devices leased in Benelux	366,6667
Number of years to break even	2,454239