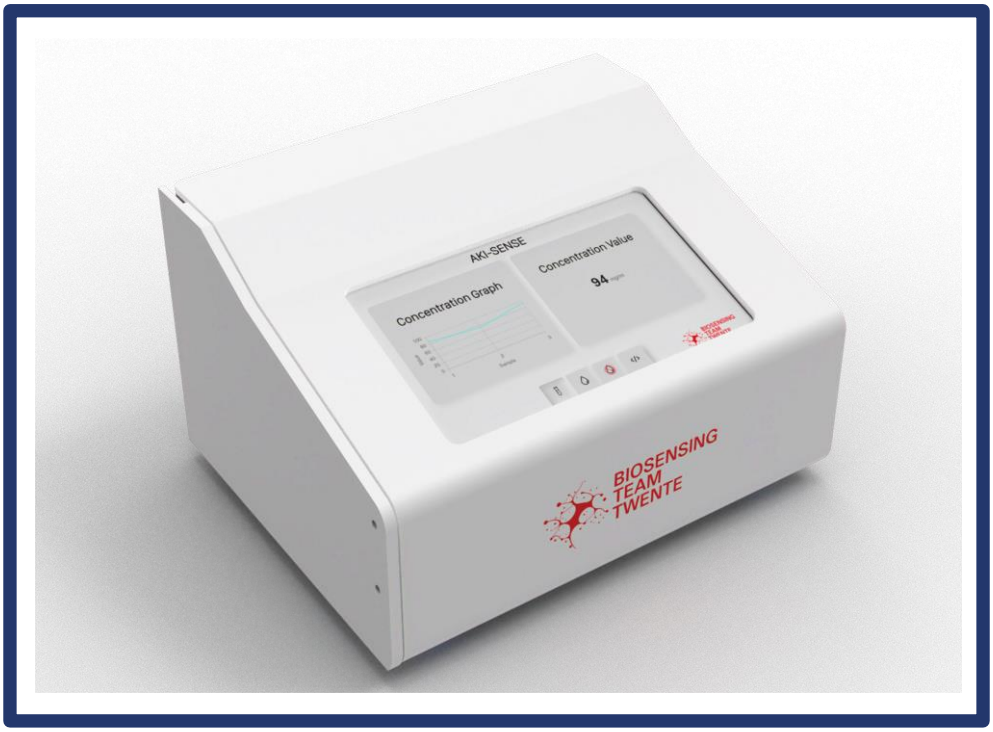
Team Results Document
Biosensing Team Twente



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9th of August 2024

SensUs

1. Abstract

AkiSense is an innovative biosensor developed for continuous monitoring of creatinine concentration for early detection of acute kidney injury (AKI) primarily aimed for ICU and post-ICU patients. Our solution aims to facilitate timely intervention, reduce hospital stays and ultimately improve patient survival while being environmentally conscious.

This is achieved through an electrochemical approach which uses aptamer-based surface chemistry, square-wave voltammetry and reversibility to create a calibration curve which allows for efficient creatinine detection. The system integrates cartridge technology with a setup containing hardware and software components for an intuitive user interface.

A translational potential case to market realization is addressed, where a 10-year business plan is outlined to reach a successful US market launch. Key considerations include stakeholder engagement, feasibility assessment and viability analyses. The central objective is to evolve AkiSense as a portable patch-system to enhance practicality, while strategically securing capital and implementing effective business strategies for successful launch, promotion, and growth.

Contents

2. Biosensor

2.1 Molecular recognition

Electrochemical sensing was chosen as the main method of sensing of the biosensor developed, due to its ability to measure specific molecules with high sensitivity and selectivity. In addition, it allows rapid response times as well as low detection limits and the possibility of miniaturization.

In the electrochemical biosensor produced, aptamers, strands of nucleic acids, are responsible for the target recognition, as these contain a selectivity and affinity towards their target-creatinine, as well as high responsiveness.

The way the electrochemical aptamer-based biosensor recognizes its targets is done with a redox molecule methylene blue, see **Error! Reference source not found.**. Upon binding of the target molecule, the aptamer will undergo a structural change causing a difference in the measured electrical signal. Due to the aptamer's conformation, the distance between the redox molecule and the electrode will be modified, resulting in a change of conductivity, thus resulting in an altered electrical signal. Different factors may influence the aptamer efficiency. The produced signal arises from the redox reaction of the molecule attached to the aptamer, meaning that factors such as aptamer density will influence the outcome. The aptamer density depends on factors such as variations in concentration, as lower concentrations may result in reduced signal intensity and diminished detection sensitivity. Thus, it is important to consider the functionalized surface and the concentration of aptamers used to maximize the Signal-to-Noise-Ratio (SNR) value. The optimal aptamer density specific to the sensor is based on the electrode surface and material since there must be enough space and conditions for the aptamers to bind. In our specific case a 3' modification with Methylene Blue (atto MB2) and a 5' Thiol functional group were chosen. (Frutiger et al., 2021; Pan et al., 2023; Parolo et al., 2023; Squires et al., 2008)

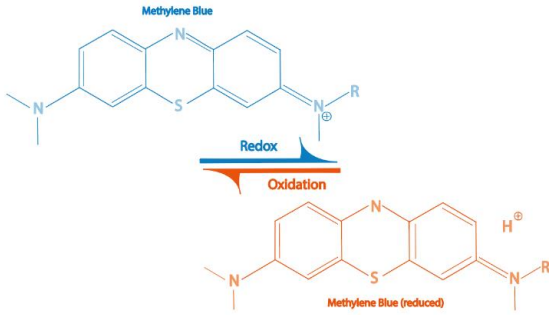

Figure 1: Redox reaction of Methylene Blue

2.2 Functionalization

The purpose of the chip's functionalization is to create a surface for the creatinine to bind, enabling its immobilization for sensing. This functionalization includes a thiol-aptamer based layer, which easily binds to the gold surface because of the high affinity. In order to increase the surface sensing area, and as a consequence the sensitivity of the device, gold nanoparticles were flown in the chip before the functionalization, just after the cleaning process.

Following the aptamer layer, a layer of Mercaptohexanol (MCH) was first applied as an antifouling layer to prevent non-specific binding and maintain selectivity. However, following experiments, MCH was discarded from the functionalization process as it was lowering the signal to a point where it was not possible to visualize the binding phenomenon. This outcome, along with the QCM-D results, are presented in Appendix IV.

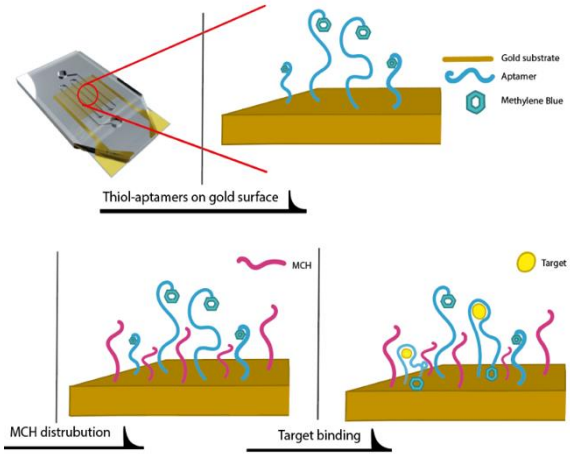

Figure 2: Chip functionalization

The functionalization was tested and validated using Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D), which detects mass changes in the sensor surface that arise from the binding and unbinding of molecules to the surface, using a standard QCM-D set-up. According to the QCM-D experiment a waiting time of 2h was sufficient to ensure binding of the aptamers to the gold surface.

The functionalization process of the final device's chips was carried out using a syringe pump, which allowed us to flow substances inside our specifically designed chips. Considering the diameters of the internal channels in the chips a minimum flow rate of 20 nL/min was used to prevent depletion during functionalization.

To check whether the process was carried out successfully, PBS was flown through the chips before and after every step, and Square Wave Voltammetry was applied to observe a difference in amplitude. The unfunctionalized chip measurement was taken as reference; after the aptamers bind to the surface, a slight decrease in the conductance is expected while after MCH we expected a substantial decrease. (Chadha et al., 2022; Gosai et al., 2016; Mirceski et al., 2018; Pan et al., 2023; Parolo et al., 2023; Warren, 2016)

2.3 Physical Transduction

The sensing method used is based on the conductance change due to the binding of creatinine to the aptamers, interaction which is then converted into a measurable signal by a transducer. Square Wave Voltammetry (SWV) was chosen as a transduction method to sense the creatinine concentration in a serum sample.
In order to choose the SWV settings, Cyclic Voltammetry (CV) was used to test the procedure was working and find the aptamers oxidation and reduction points. Being aware of these values allowed us to find the proper working range to perform the SWV and not break down the functionalized electrode's surface.
SWV is a specific type of pulse voltammetry, that minimizes the capacitive background current of the working electrode, whilst maximizing the signal-to-noise ratio of the Faradic currents of the oxidation and reduction waves.

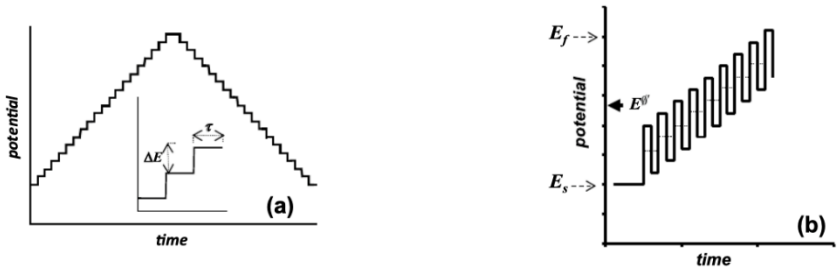

Figure 3. SWV voltage input over time

In order to ensure continuous operation, the biosensor must incorporate a method for the controlled release of bound creatinine molecules, known as unbinding. This can be achieved by natural phenomena or alternatively, by applying a suitable electrical impulse to facilitate the release process. This method not only ensures real-time monitoring but also maintains the integrity of the biosensor chip throughout the process, thereby enhancing its reliability and usability.
As reported in Gosai et al. Experiments, a positive electrical stimulus of within a range of 0.5 - 1 V is sufficient to influence a protein-ligand interaction without modifying their characteristics.
During our experiments we found out that 0.5 seconds lasting impulse was changing the signal and resetting the device for the next measurement, but more experiments should be done to optimize the process.

2.4 Cartridge Technology

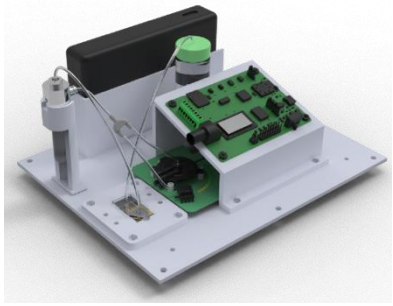

Figure 4. Device Set-up

The biosensing device consists of a flow cell that splits the sample in two, pushing it with a piezoelectric pressure pump into a sensing arm and a reference arm. The chip is made in glass – where gold interdigitated electrodes are located – and PDMS (Polydimethylsiloxane) to shape the microfluidic channels. The electrodes have overall dimensions of 14mm x 17mm, and the channels of 300ul x 250ul.
As shown in **Figure 4**, the sample is manually inserted into the vial, which is connected to the pressure pump and to the chip. The vial with the sample is stabilized by a holder, and the vial can be removed to insert the sample. The chip is stabilized in a holder and connected to the waste collection. The electrodes of the chip are connected to the potentiostat, which together with the pump is connected to a Raspberry Pi.

2.5 Reader Instrument & User Interaction

The device comprises of an external housing and internal components. The housing, constructed from eco-friendly PLA plastic using 3D printing, provides easy access to internal components for maintenance and upgrades. The Raspberry Pi 4 serves as the device's central processing unit, managing data flow between the user interface, sensors, and actuators. Key components include a potentiostat module, pump, sample vial, sensor chip, waste bottle, and power bank.

The user interface is designed for straightforward operation, primarily focusing on data display. A concentration graph and numerical value provide real-time measurement results, as shown in **Figure 5.** Basic controls allow for sample input, flow rate adjustment, and access to a developer-oriented debug console.

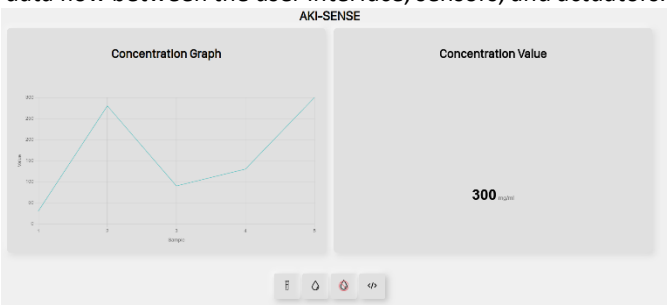

Figure 5. User Interface

3. Technological feasibility

3.1 Molecular Recognition

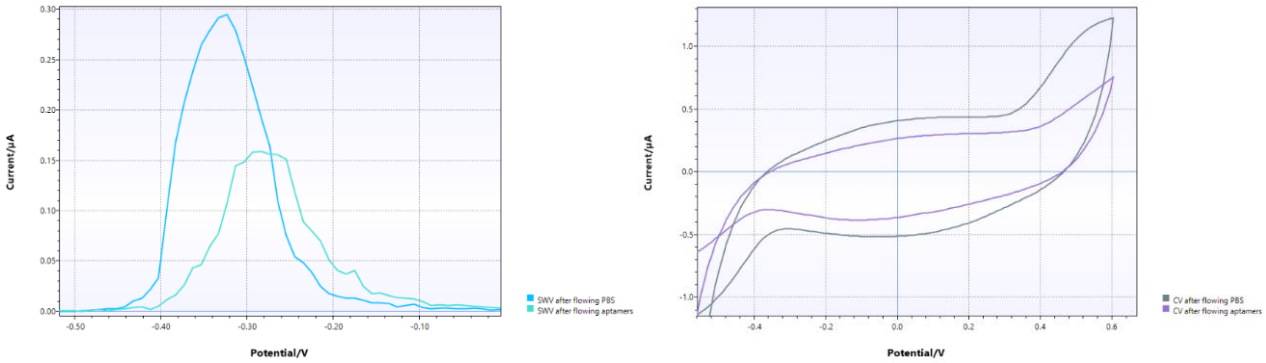

Figure 6. Functionalized chip with intermediate measurements done by a) Square Wave Voltammetry (SWV), on the left; b) Cyclic Voltammetry (CV), on the right.

The chips were initially prepared by functionalization of the surface. As previously explained, this was done by cleaning the surface, flowing gold nanoparticles, then PBS, followed by flowing the aptamers, and then PBS again. The results from the functionalization process can especially be viewed in **Figure 6a**, which displays results from SWV. The two measurements performed were obtained while PBS was present in the chip and show a difference before and after the binding of aptamers to the surface. A change in the value of the peaks can be observed, meaning that the aptamers in fact bind to the electrode surface. The CV, **Figure 6b**, shows the classic duck shape, which, as predicted, gets thinner after the surface functionalization. With these results, we can conclude that the functionalization process works effectively.

Next, several samples – diluted serum with creatinine concentrations of 30.0, 97.5, 165.0, 232.5 and 300.0 micromoles per liter - were flown into the same chip, alternated by a washing step with PBS, in order to verify whether the biomarker would correctly bind to the aptamers, and if the signal could be observed successfully.

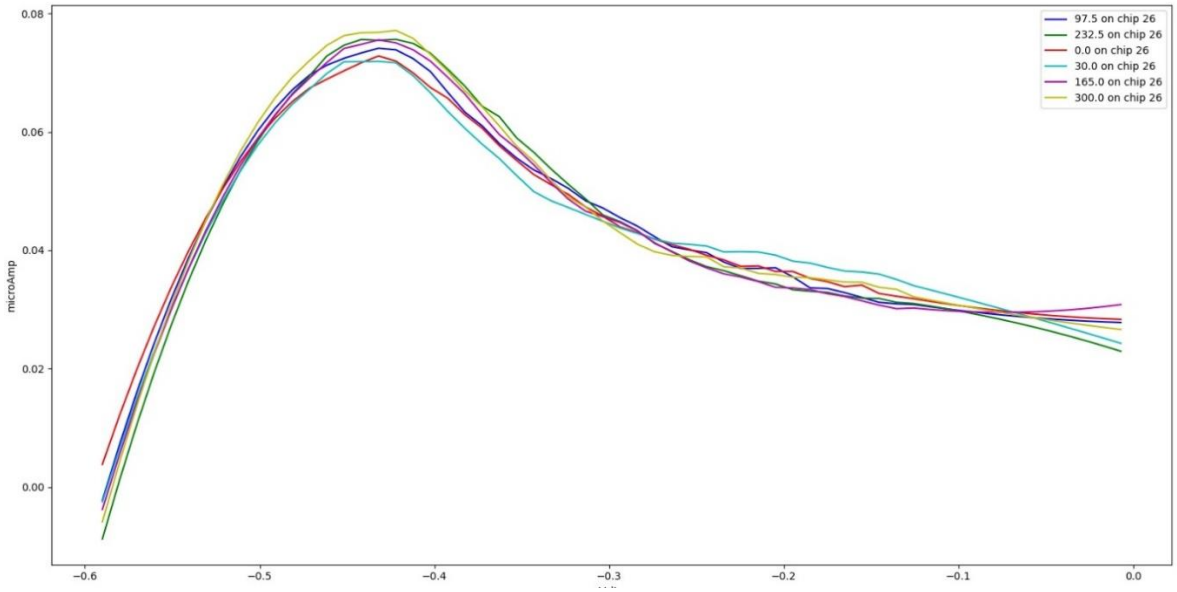

Figure 7. Results obtained from various samples with SWV, plotting current (microamperes) against Voltage (Volts).

The results are displayed in **Figure 7**, clearly showing a peak around –0.4 V, proportionate to the creatinine concentration in the sample. It can be noted that the baseline (ISF sample with no added creatinine) has very similar values to the lowest concentration sample, however, experiments to determine the lower limit of detection (LOD) of our device were not performed, as they were not necessary for our study. In the future it would be interesting to determine both Lower and Upper limit of detection.

3.2 Physical Transduction

The results shown in Figure 7 were then used to obtain the calibration curve shown in Figure 8.

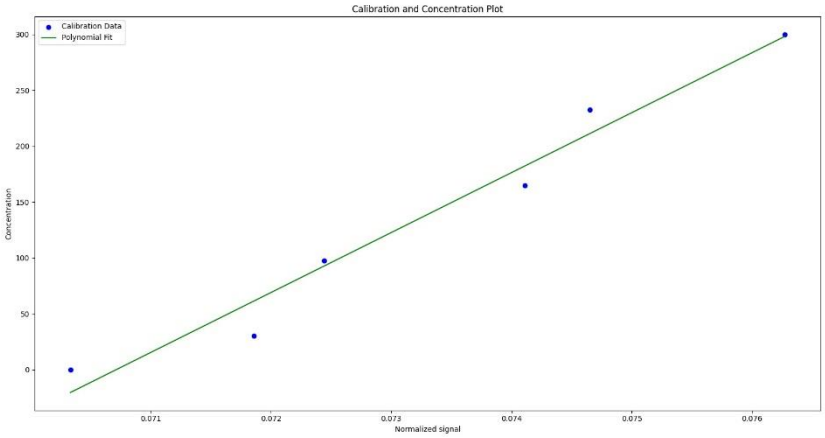

Figure 8. Calibration Curve, plotting the concentration values against the normalized current signal.

Linear regression was applied to the current values at –0.45V. The voltage value at which the current is measured was chosen based on the results shown in figure 7. It was assumed that –0.45V was the optimal voltage value to use for calibration for the specific chip, however calibration would need to be performed anew for any chip, as each one gives differently shaped results, and therefore different optimal voltages. It was in fact noted, based on repetitive experiments, that calibration curves can deviate slightly among different chips, therefore for a reliable calibration curve with clear error limits, many chips need to be exposed to the same protocol and data analysis. Additionally, the limited number of datapoints makes it necessary to perform additional measurements, in order to make it more accurate.

3.3 Fluidic Cartridge

During the fabrication of several chips, leakage was observed at the connection point between the tubes and the PDMS. This was solved initially by using glue, which was later substituted with PDMS drops, which proved to work more effectively. Additionally, the electrodes were initially connected to the potentiostat through soldered cables, but the results obtained were too noisy. Therefore, thinner, single-strand cables were used, which were connected to the electrodes using silver paste

3.4 Reader Instrument

The process of reading the sample starts with inserting a vial with a new sample into the pump, then the user can initiate the measurement via the user interface. The sample is pumped through the cartridge using a pressure pump, after the sample has completely covered the chip, flow rate is decreased to perform measurement. After the measurement is complete, the user is prompted to replace the sample with a vial with a new sample. The unbinding step takes place before the measurement, while the device is pumping the sample to the cartridge, if it is not the first measurement.

To be able to clean the components of the device from inside after all measurements are complete, the user can initiate flushing, during which it is possible to start and stop the pump.

4. Originality

4.1 Team Piece

Our team initiated the biosensor development process with a comprehensive literature review focusing on conformational switch sensors, recognition layers, surface chemistry, electrochemical techniques (interferometry, cyclic voltammetry, square-wave voltammetry), and the advancement of current surface-based biosensors. Through weekly discussions with researchers working on these topics, we identified aptamer-based surface chemistry as the most promising approach for our biosensor development and focused our efforts in this direction. By doing so, we could take advantage of the support and in-house expertise within our university.

Our device combines different traditional techniques to effectively measure creatinine concentration in a sample. Thiol-aptamers on gold is a well-established technology, which ensures binding between the electrode surface and the aptamer. However, the use of aptamers allows us to reverse the binding between the aptamer itself and the biomarker. This is performed by sending electrical impulses on the surface and makes it possible to achieve an effectively continuous measurement, without the use of cleaning steps which would affect the quality and the functioning of the final device. The sensitivity is additionally increased by adding gold nanoparticles on the surface, which increases the sensing area.

A focus was placed on creating a user interface that facilitates usability and that accounts for the reader instruments and overall design. User-friendliness and simplicity were prioritized when iterating different versions to enhance user experience. This will be adopted going forward to the smart patch minimal system that is a wearable, as depicted in the business case. The current version uses a Raspberry Pi, a touchscreen, a micropump, a chip and a waste container to run the entire setup in an efficient manner.

Danail Tsanov

Caterina Cattabriga

4.2 Supervisor Piece

The team began their journey by comparing literature review possibilities with the expertise available within the University of Twente. They looked into electrochemistry, photonics, and MEMS-based assays in line with the expertise available at the UT groups.

This year the UT SensUs team has become an official student team. This has helped to link with last year's core team, which formed the board for the student team. Based on the know-how available from last year, its high sentivity and selectivity, and versatility, the team decided to use electrochemical sensing, combined with aptamer based biosensing. This choice allowed the team to build upon existing networks and expertises such as the Bioelectronics group (BE), the Molecular Nano Fabrication group (MNF), the Lab-on-chip group (BIOS), and the Robotics and Mechatronics group (RAM). The team worked autonomously in setting up their development plan. They had however a big setback due to the delayed delivery of the aptamers over three months. This had a considerable impact into the development plan. Mohammad Saghafi provided supervision for the electrochemical measurements, while Nico Overeem was asked for advice on surface chemistry, and Pep Canyelles Pericàs focused on mentoring the business case and team coordination. The team benefited also from coaches from different groups.

Pep Canyelles Pericàs

5. Translation potential

5.1 Introduction

Acute Kidney Injury (AKI) is a worldwide disease that leads to high mortality rates (22-67%) especially among intensive care units (ICU) patients and can occur up to 30 days after surgical intervention(Bell et al., 2017; Trongtrakul et al., 2019). Early detection and intervention are crucial for improving patient outcomes and reducing healthcare costs. Our device, AkiSense, is designed to address this need by providing real-time monitoring of kidney function via creatinine concentration. By enabling early detection, we aim to facilitate timely intervention, reduce hospital stays, and ultimately improve patient survival rates.

The US market, represents a significant portion of the global point-of-care biosensor market (approximately USD 2.2 billion compared to Europe's USD 1.35 billion), offers a favourable landscape for AkiSense's launch due to clear regulatory guidelines, a larger market size, and a rich investment ecosystem. While the European market holds potential, regulatory complexities across different countries leave this as a future growth opportunity to that of the US (Fink & Akra, 2023). The platform technology could extend further than AKI, with potential applications in monitoring other critical biomarkers such as cardiac troponin for early detection of heart attacks, enabling the device for wider applicability. Further detail is provided in the Lean Canvas in **Appendix I**.

5.2 Stakeholder desirability

5.2.1 Customer

The main target are hospitals in the US that want to reduce costs by sending patients home earlier, while maintaining the same quality of care provided or even higher by continuously monitoring the creatinine levels in the interstitial fluid of the patients. Insurance programs in the US, such as MediCare, will play a crucial role in this approach, as they will have to pay for the reimbursement of the device. Enough clinical evidence and innovation value in the product needs to be proven to be in the list of reimbursed devices, which will ensure that the hospitals costs for the patient are covered by the insurance company.

5.2.2 Stakeholder and their needs

Insurance companies- MediCare is a health insurance program in the US, their requirements are to ensure that it provides effective and efficient coverage to its beneficiaries, for our device to be aligned with these requirements it needs to be a cost-effective solution, safe for the patient and innovative enough to be added to the reimbursement program.

Hospitals- Ochsner LSU Health Shreveport - St Mary Medical Center, that performs around 240k major surgeries a year (*Top Hospitals Performing the Most Surgeries in the U.S.*, n.d.) and therefore will benefit greatly from reducing patient time in ICU, need to provide quality healthcare to the patients while maintaining operational efficiency, one of the methods to achieve that is by adopting new technologies, but only when the cost savings are clear while improving patient outcomes.

Patients-Patients expect fast diagnosis, effective treatment and early discharge in case of hospitalization. They expect healthcare solutions that can quickly identify any health issues to prevent complications and enable early intervention. Comfort and minimal invasiveness during their medical care are also important, as they want to reduce the stress associated with diagnostics and monitoring after discharge from the hospital.

Research and Development- For the device to be ready for clinical trials there needs to be a miniaturization of the current prototype. Our partner Benchmark, expert in medical device solutions will develop the new device, while PalmSens will help us design a robust biomarker chip technology to ensure precise monitoring. BAAT Medical will guide the development of the device to make sure that it complies with the required regulation.

Expertise- During the last year we've interviewed (**Appendix XI**) and built a network of connections with hospitals and companies, like prof. dr. Laverman from ZGT Hospital, PhD Christina Gant UMC Utrecht, prof. Stamatialis, head of Advanced Organ Bioengineering at UT TechMed Centre in the Netherlands and with MD Lluís Guirado, nephrology director from Fundació Puigvert in Barcelona, companies like BAAT Medical (experts in regulation), Benchmark, Demcon and PalmSens (manufacturing of medical devices), that will provide guidance throughout the entire development phase.

Clinical trials- Clinical trials will be conducted in the University of Chicago, as they are experts in this type of studies with biomarkers in renal diseases and are based in the US. The initial reaching to the university will be conducted by using the academic network of the University of Twente and further relationships will be developed over time.

5.2.3 Outline of the qualifications of profitable customers

Hospitals that benefit the most will be the ones that have patients in the ICU who have undergone major surgeries or have developed septic or cardiogenic shock, hypovolemia, hepato-renal syndrome or obstructive uropathy, which make up 5-6% (Uchino et al., 2005) of the 5.7 million ICU patients a year admitted in the US (Viglianti & Iwashyna, 2017), which means around 300,000 patients a year might benefit from using the device. These conditions are major contributing

factors for developing AKI in ICU settings, and having a solution that can reduce the time spent in the ICU is very attractive to these hospitals.

5.2.4 Added customer value of product

The hospital will benefit of early detection of AKI, preventing further complications, having the ability to monitor creatinine levels continuously to make timely adjustments, leading to fewer resources allocated for each patient using the device.

5.2.5 Rules and Regulations

To achieve regulatory compliance in the US the device needs to comply with the Protected Health Information (PHI) regulations under HIPAA, and FDA approval. The company must comply with Quality System Regulation (QSR), ensuring that the company has a quality management system in place covering design control, production, processes, process validation, complaint handling and respective ISO standards. Further detail is provided in **Appendix III**.

5.2.6 Value Proposition

Competitors

We distinguish between two types of competitors, general medical device companies that have the resources to develop such a product and specific AKI diagnostic companies.

General Medical Device Companies:

- Roche Diagnostics: Offers a range of diagnostic solutions including blood and urine creatinine testing.
- Siemens Healthineers: Provide diagnostic equipment for monitoring kidney function.
- Abbot Laboratories: Have a point of care system called i-STAT 1 that can measure biomarkers from the blood, which can potentially be adapted to monitor creatinine levels.
- Baxter International: They have products focused on renal care.

Specific AKI diagnostic Companies:

- RenalytixAI: Assessment of risk for progressive decline in kidney function, mainly focused on diabetes patients.
- BioPorto Diagnostics: NGAL biomarker detector in urine. Focusing on pediatric patients.
- MediBeacon: Kidney function measurement technology, using transdermal fluorescence.
- RenalSens: Monitoring of early AKI by using urine flow.

Comparison with product

Table 1: Competitors product comparison

Product	Technology	Target	FDA Approval	Monetary Valuation
RenalytixAI	Blood biomarker detection	Diabetes type 2 patients	Yes, included in MediCare	17.1M (*Renalytix AI Plc ("RenalytixAI" or the "Company") Full Year Report - Renalytix*, n.d.)
BioPorto	NGAL in urine flow	Pediatric ICU patients	Yes	11.9M (BioPorto, n.d.)
MediBeacon	Transdermal fluorescence	ICU patients	No, clinical trials ongoing	400M (Kovesdy, 2022)
RenalSense	Urine flow	ICU Patients	Yes, and CE	14M (*RenalSense Company Profile 2024: Valuation, Funding & Investors | PitchBook*, n.d.)
AKI-Sense	Continuous transdermal needle creatinine monitoring	ICU patients	No	See **Appendix VI, VII**

Analyzing **Table 1**, all the devices focus on kidney monitoring, most of them target ICU patients and aim for FDA approval, which indicates that we are aligned with the competition. The main difference is the use of a novel technology, a different biomarker and being the size of a small patch, which gives us a competitive edge on the market.

Intellectual property of competitors in relation to product

- RenalytixAI: 3 proprietary blood-based biomarkers, validated by third-party studies.
- BioPorto Diagnostics: Initially founded to commercialize intellectual property licensed from the Statens Serum Institute.
- MediBeacon: Patent portfolio including more than 30 patents related to the composition of matter, algorithm and method of fluorescence, in the US, Europe and globally.
- RenalSense: Patented electronic sensor for the Clarity RMS system.

Outline of the degree to which the prototype lives up to the benefits described

The prototype has the capability to continuously monitor a patient but only in the ICU setup, it can be operated automatically and has a friendly UI for the medical staff, indicating the progression over time of the concentration of creatinine and the ability to make changes to the different parameters of interest or display more technical information in case is needed. The precision of the device is yet to be determined, but the initial results obtained in the Distributed Testing Event are promising, aiming for future iterations to monitor creatinine levels withing a clinically acceptable range of 30-300 µmol/L. The processing of the data is done on-device, meaning faster results compared to a cloud-based solution. The device can connect to a cloud database, so the medical staff can access the data anywhere.

5.3 Business Feasibility

5.3.1 Key resources

Specialized Equipment

The biosensor's development required specialized equipment that included and was not limited to chips, electrodes, reagents, aptamers, PDMS and PDMS molds, 3D printers and filaments, soldering equipment, wires, LCD, Raspberry Pi, laser cutting equipment, plasma gas machine, sonicators, desiccators, an oven, fridge holepunches and basic lab tools. To transition from our current, benchtop prototype to a wearable device, we will partner with established companies like Benchmark or PalmSens for manufacturing and production in the distant future. This will require additional resources for storage, shipment, and staff training. Successful and timely US market entry requires navigating FDA regulations. We will leverage university resources and partnerships (e.g., NovelT) to access experienced professionals familiar with the approval process. A detailed breakdown of our market entry strategy is provided in **Appendix III**.

Know-How

We collaborated with research groups from our university to optimize protocols, reduce waste, and improve efficiency. Specifically, we learned from the Molecular Nanofabrication group about surface functionalization, the BioElectronics group provided insights into electrochemical sensing, the Robotics and Mechatronics group guided us on interfacing and control, and the BIOS Lab on Chip group shared expertise in microfluidics and lab-on-chip systems.

Human Capital

Our team had a strong base in biomedical engineering, complemented by industrial for production optimization, communications student for outreach and computer scientist for data analysis. However, the needed specialized areas like surface chemistry, microfluidics, regulatory compliance, business analytics and legal advice were found through our university network and team connections. Further validation on our approach was needed and gathered through interviews with medical practitioners, business analysts and legal advisors to ensure the device's functionality, market fit, and regulatory compliance. Details on these interviews can be found in **Appendix XI**.

Wider UT Support

Our successful venture with Techmed Centre, Design Lab at the University of Twente and our sponsor Benchmark provided the funding needed to purchase the necessary materials. A breakdown of the resources skills and expertise is provided in table matrix found in **Appendix II.** Further collaboration with NovelT will get the business started and then will follow the path outlined in **Appendix III**.

5.3.2 Key activities

Figure 5 outlines the phases, key events and activities that are needed over the span of 10 years. A breakdown of each phase and the sub-activities is provided in **Appendix I**.

To transition from a student team to a company, we will leverage NovelT, the university's Knowledge Transfer Office (TKO) for legal, financial, and startup support. This will involve strengthening our relationships with their patentability, business development, and market analysis teams. Afterwards, adherence to different International Organization for Standardizations (ISO) must be complied to build credibility, have efficiency and secure market access to certain regions. A list of all the relevant ones is provided in **Appendix III**.

Further research and development are necessary to optimize our device and allow it to be used as a wearable that can project data to the user and practitioner through an application, similar to a glucosensor. Partners, like our sponsor Benchmark or PalmSens, will be used to achieve this.

Our marketing strategy involves a multi-faceted approach, combining direct sales, targeted outreach, and digital marketing. We will prioritize healthcare institutions with high AKI prevalence, such as Intensive Care Units (ICUs) and surgical wards. Building strong relationships with key decision-makers in these settings will be crucial for early adoption success. We will participate in industry events, conferences, and workshops to showcase the product's value

proposition. Our digital marketing strategy will focus on content creation and distribution. We will collaborate with relevant scientific and health publications to increase brand visibility and highlight the device's benefits.
Our revenue strategy involves licensing to companies, as outlined in **Appendix II**. We will focus on a single strong patent, specifically targeting the functionalization of our aptamer for creatinine, as advised, by the patent advisor Niels Jansen from NovelT. This approach balances robust protection and financial feasibility. After conducting a patentability search and filing for an international patent, we'll pursue non-exclusive licensing agreements with companies across different regions. This allows us to generate revenue while retaining control over technology use.

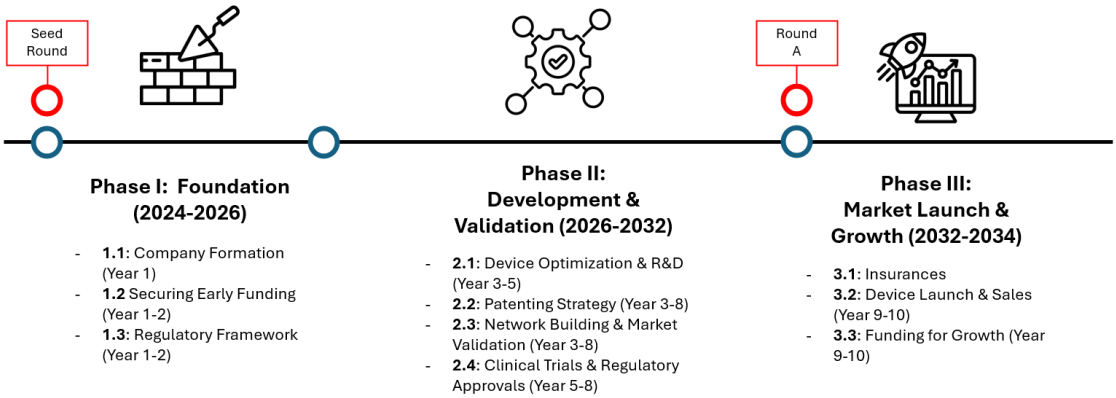

Figure 9: Business Case 10-year Timeline

We will secure the necessary capital for further development and marked entry through a phased ABC series model which will allow for strategic adjustment during milestone progression. The initial Seed round of funding will focus on the development activities outlined in the timeline of Phase I and II **(Figure 5).** **Appendix III** provides a detailed breakdown of potential funding sources. Our focus will be on subsidies and grants to avoid diluting ownership. Options like the Open Mind Grant (€50,000), the Take Off Grant (up to €250,000) and SME Innovation simulation region and top sectors will be a starting point.

5.3.3 Key partners

Research Collaborators- **Molecular NanoFabrication, BioElectronics, Robotics and Mechatronics, and BIOS**
These research groups offer specialized facilities and expertise in microfluidics, surface chemistry, and biosensor development. In return, we provide access to our biosensor technology, potential for joint research projects, and opportunities for student internships.
Manufacturing Partners- **Benchmark, PalmSens, Uneedle**
These partners contribute manufacturing capabilities, device miniaturization, and software development and in return we will explore potential licensing agreements and collaborate on joint product development.
Business Support – **NovelT and Incubase**
Both will provide legal, financial, and startup support, and networking opportunities. In exchange, we will offer access to our innovative technology and the possibility of equity investment.
Funding Sources- **TechMed Centre, Design Lab, venture capital firms and government grants**
We will work with different funding sources, to gain financial resources, mentorship, and industry connections. In return, we negotiate equity arrangements, intellectual property rights, and the potential for higher returns.
Regulatory Bodies- **FDA ,CE, Regularory Marking authorities**
These partners will have guidelines and approval processes. We will offer compliance and in turn this will permit us for secure market access depending on the geographic region.
Healthcare Providers: **Hospitals, Clinics, Medical Practitioners**
These partners serve as clinical testing sites, provide patient feedback, and act as early adopters.
In turn, we gain access to real-world data and opportunities for collaborative research.
Industry Associations: **Renal Healthcare Associations and Medical Device Industry Associations**
These associations will offer us industry insights, networking opportunities, and policy advocacy. What we can offer is participation through membership fees, engagement in industry initiatives and to raise awareness of AKI treatment.
For further details, refer to the Key Partner Rubric found in **Appendix V**.

5.3.4 Sustainability

Sustainability is a core principle driving our biosensor development. We used expertise from university researchers who are familiar with the labs to optimise our protocols and chemical usage and energy exposure while working. Resulting in the introduction of circularity principles by reusing lab equipment and waste generation reduction. The continous nature of our device promotes sustainability by eliminating the need for single-use options and will reduce healthcare

material waste. Our next phase that focuses on minimizing the design will reduce the materials needed for production. After this stage, we will be introducing end-of-life plan that includes recycling and disposal options for the device and padding system. We will also prioritise partners, like Benchmark, who use sustainable waste management and are aware of transportation and packaging solutions to decrease our carbon footprint. Adherence to local, national, and international sustainability regulations will ensure our practices stay within the legal framework for the region of operations. For example following the directive 2008/98/EC (Directive - 2008/98 - EN - Waste Framework Directive - EUR-Lex, n.d.) within Europe and the RCRA (Resource Conservation and Recovery Act) in the USA. (Regulatory and Guidance Information by Topic: Waste | US EPA, n.d.)

5.4 Financial Viability

5.4.1 Costs projection

Category	Investment, (€)
R&D Expenses	2,000,000
Regulatory Compliance	2,000,000
Intellectual Property (IP) Protection	500,000
Manufacturing Setup	800,000
Marketing and Distribution	200,000
Total Cost (M), EUR	5,500,000

Table 2: Cost Projections and Seed Round capital goal

A comprehensive overview of the device's finance projections is provided in **Appendix VI** which considers the 3 phases as explained in **Appendix III** and is shown in **Figure 9**. External support to construct this was gained from the TKO expert, *Tim Jungman*, from our university. **Appendix VII** shows realistic costs of development and implementation of the current version per device- Sensor Material 303.3€ and Chip Costs 121.2€. The current major cost can be decreased by minimizing the Power bank and LCD screen which will not be needed for the envisioned smart patch and phone app product. It is believed that bulk production will lower manufacturing and assembly costs by a 25% margin. Further cost projections are depicted in **Table 2,** in addition descriptors of the categories is provided in **Appendix X**.

5.4.2 Sales price

Our device can be broken up into the Cartridge and the Sensor. Respectively each has been calculated to cost 303.3€ and 121.2€, details of which can be found in **Appendix VII.** Additional costs such as packaging, transportation, labour costs and insurance bring the device to 39€. Together our current iteration of the biosensor comes to a total of 463.5€. This accounts for the current version of the device, however following the R&D in Phase II, the1 sales price is certain to rise higher to at least, accounting for an acceptable profit margin comparable to market standards of 539€. The future device will bring extra revenue from the patches bought by the user (3.5 € for pack of 3 with life cycle of 7 days each) and by the standard (5.99 € per month) vs the premium (9.99€) accounts. Detailed account is given in **Table 3**.

Table 3: Revenue Streams Sales Price Breakdown

Revenue Model	Total Revenue (€)	Cost of Goods Sold (COGS) (€)	Gross Profit (€)	Gross Profit Margin (%)
Device	539.00	463.50	75.50	14.00%
+ Standard Subscription	610.88	463.50	147.38	24.13%
+ Premium Subscription	658.88	463.50	195.38	29.65%
+ Patches (Standard)	669.50	463.50	206.00	30.77%
+ Patches (Premium)	717.50	463.50	254.00	35.40%

5.4.3 Market analysis

Our primary target is launching in the US and then targeting other locations such as the EU. Annually there is an estimated incidence rate of 50.8 million cases where AKI can develop, which accounts for cardiac surgeries, internal medicine, urology and nephrology. The US market was chosen as it offered the largest market share worldwide, had clear regulatory guidelines (FDA) and due having large support frame for new MedTech innovations. It could potentially bring in revenue of an estimate of 154 million euros, as explained in **Appendix VI**. The global market for biosensors is determined to grow with a compound annual growth rate (CAGR %) of 7.7%. The USA in particular with a CAGR of 4.1% (Acute Kidney Injury Market Size, Share, Therapies, Key Companies, n.d.). This shows steady increase to the near future, due to aging populations and growth of developing countries. The burden of chronic diseases will only drive interest in the development and improvement of preventative healthcare solutions similar to our AkiSense.

The current golden standard biosensors are the gluco-sensors used to monitor diabetics. Out of them, notable leaders in the US market are Dexcom's Stelo Glucose Biosensor and Medtronic's Guardian system. Current continuous measuring devices for AKI devices were not found during our investigation, meaning that we will be one of the pioneers in this segment. The aim is to reach a 3% of the market share by the end of our Phase III which entails to have at least

one of our devices in 180 locations out of the total 6120 hospitals in the US. This accounts to roughly reaching 1.5 million cases, which would bring a revenue of a 100 million euros (profit margin of that would be roughly 30% if prices remain unchanged). A focus will be made to target major institutions that specialise in surgeries and have large ICU units as mentioned previously. We believe that other institutions will capitalise on the results that our solution provides and on the reduced hospitalisation duration, creating a snowball effect. This was supported through the interviews that we carried out with healthcare practitioners, who noted that this would be the case if the device lowers costs of healthcare and provides better outcomes for patient.

The US market holds a significant promise for financial growth and the 5.5 million as requested from the Seed Funding round and shown in **Table 2** will be sufficient to drive the development of our device as shown in **Figure 10.** The development costs will impact initial growth in the early years but from the break-even point in year 8 (2031) onwards, revenue generation is prognosed. Detailed costs breakdown is provided in **Appendix VI**. After establishing steady sales and growth in the US, expansion to the EU will begin, which would close to double the total market space.

5.4.4 Revenue streams and business strategy

Our primary revenue stream will come from a (i) **subscription-based model**, offering tiered access to data analysis features to our base model (5.99€/month). A premium subscription level (9.99€/month) will provide advanced analytics, detailed reports, data exportation, early access to new features, additional features like creatinine level tracking, warranty options, and software/hardware customisation. To incentivize early adoption and bulk purchases, we will implement tiered pricing structures and promotional packages. In addition **(ii) smart patches** (3.50€/month) will be needed to keep the device functional and secure.
(iii) **Data monetization** (15€ per data point) will be explored through the anonymized sale of aggregated data to pharmaceutical companies, research institutions, and government bodies, while adhering to strict ethical and regulatory guidelines. To complement subscription revenue, we will consider direct device sales. A combination of these revenue streams will contribute to the overall financial sustainability of the business. Until market launch the main income will come from strategized (iv) **non-exclusive licensing agreements** (20000€ per licence) in different geographical regions. A clear comparison between the expected costs of development and revenues during the next 10 years is provided in **Appendix VI**.

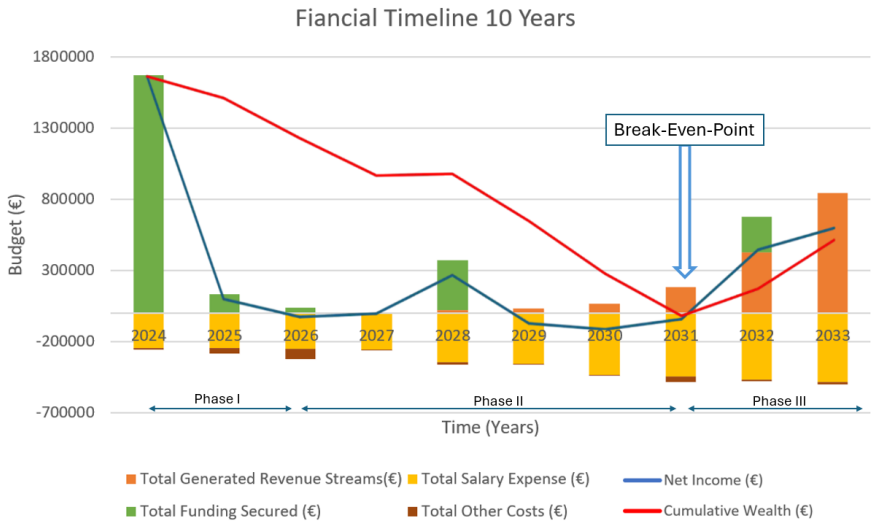

Figure 10: Chart displaying Fund Allocation and Break-Even Point

5.4.5 Business Intelligence Set-up

Our business intelligence revolves around first gathering data from many sources such as on the sales, user behavior, device performance and internal production. Then this information is to be processed and analyzed to identify market trends, growth/decline opportunities on user preferences for optimization and operational efficiency. Relevant key performance indicators (KPIs) will be assigned and will then be addressed based on their priority and or urgency. We aim to use business intelligence to make data-driven decisions to ultimately promote business growth.

6. Team and support

6.1 Contributions of Team Members

Danail Tsanov- Captain of the team. Involved in team management and coordination, Chip Design for PDMS fabrication and protocol writing, Device Design for idealizing the concepts and with a focus on the Business Case development.
Caterina Cattabriga- Captain of the team. Involved in team management and coordination, with a focus on the coordination and planning of the work of the Chemistry, Chip Design and Experiment and Analysis subgroups. Took part in the planning and execution of experiments both in the Chemistry and Experiment and Analysis subgroups, and took part in the designing and fabrication process of the concept for the Chip Design subgroup.
Alex Faloppa - Team member involved in multiple sub-teams with a focus on Experiments and Data analysis. Took part in the chips fabrication, functionalization and testing. The main activities included building a basic setup to run the experiments, functionalizing the chips, and various experiments to test the unbinding of creatinine and the sensitivity of the device. Eventually assembling the final prototype of the device.
Hanna Lücking - Team member involved in the Marketing & Communication and Business Case subteams. Responsible for creating content and posting on our social media accounts on Instagram and LinkedIn, writing press articles, designing merchandise, team shirts and advertisement products such as flyers and banners. Developing our business model and presenting during entrepreneurship sessions.
Pelin Akca – Team member involved in Experiment and Analysis, Chemistry and Device Design. In early stages of the team, took part in literature research regarding the surface chemistry. For the experiments, took part in the fabrication of the chips, functionalization and working with the pressure pump. And lastly, took part in consulting the designers to create the correct setup. Also contacted the company that delivered the aptamers, since these were very delayed.
Vinaya Tennakoon – Team member involved in the Chemistry and Experiment and Analysis team. Took part in literature search for the surface functionalization and worked on the QCM-d to analyze different characteristics of the available functionalizations.
Marisa Paiva Silva- Team member involved in the Chemistry and Experiment and Analysis team. Took part in literature search for the sensing method as well as in the QCM-D experiments to analyze different functionalization methods. Furthermore, was also involved in the business case, specifically in the Business Feasibility section.
Ken Heng – Team member. Involved in multiple sub-teams. Main team designated is Device design by working on device design ideation of exterior and interior place of components, and prototyping and finishing of device. Other than that, Chip design with refining PDMS protocol and fabrication work. Social media working on revamping the main website, capturing and editing photos used for social media platform post, designing flyers, banners and team shirts and contacting vendors to order team merchandise.
Xavier Mourelo Garcia – Team member. Involved in the chip design team, both the electrodes and PDMS channels design. Involved in the device design team, providing general guidance on the design and developing the chip holder system.
Julia Paredes - Team member. Involved in early literature reseach in the Chemistry team regarding the unbinding of the aptamers and with main focus on the Experiment and Analysis team. Took part in the literature search for the methods for testing and worked in the laboratory for the fabrication of PDMS and the fabrication, functionalization and testing of chips.
Janine Ferro- Team member. Involved in Chip Design subgroup with prime focus on design and manufacturing of the chip used and flow splitter.
Danila Bren- Team member involved in Experiment and Analysis and Chemistry subgroups. Responsible for developing code, carrying out lab work and analysis of results.

6.2 Externals Contributors and Additional Support

Dr. ir. Pep Canyelles Pericas - Team supervisor who held weekly meetings to ensure the team was on track and cohesive, main focus was supporting the and supervising the development of the Business Case.
Dr. ir. Mohammad Saghafi - Lab activities supervisor, supported us in developing the necessary techniques and understanding required to proceed with the device; held stand-up meetings to track and provide feedback.
Amarna Pels – Chemistry expert supervisor. Support in the use of QCM-D machine, including development of protocols, planning of experiments, and interpretation of results.
Daniël Wijnperle – Supported the team during the electrode and moulds fabrication process in the clean room.
Dr. ir. Nico Overeem – Contributed in the ideation of the biosensor and understanding of its functioning mechanism and chemistry.

7. Final Remarks

We would like to thank everyone who has been involved in the development of our device for their insights, time and efforts to help us grow both academically and as people throughout the past 8 months. This of course includes also the SensUs team and Jury who acted also as a support frame for our development with the multiple feedback sessions and other events.

We are proud to say that we have surpassed our initial expectations and pushed through all the challenges we faced to get to this point with grit and saw growth in many fundamental areas such as team-work, creativity and resilience. Our diverse educational and cultural backgrounds enriched, not only in meeting the technical goals that we outlined for ourselves and brought different perspectives to discuss, but also in having fun along the way.
We are sure that through our device we have addressed and provided a viable healthcare solution to AKI monitoring, which despite it needing further polishing up is a great start which has feasible future aspects.

We are grateful for the opportunity to participate in the SensUs competition and would like to extend deepest appreciation to our supervisors. Dr. ir. Pep Canyelles Pericàs and Dr. ir, Mohammad Saghafi, and the supervisory board who have been support beacons since the beginning of the year.

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9. Appendix

Appendix I: Business Lean Canvas Model

Lean Canvas

Designed for:	Designed by:	Date:	Version:
People in need of continous test results for AKI marker	Biosensing Team Twente 2024	17.07.2024	4

Problem

Reliance on laboratory testing protocols causes significant **delays in diagnosing** acute kidney injury (AKI). **Increased medical expenses** due to these delays lead to higher medical costs and prolonged hospital stays. They are ultimately resulting in **worsened patient outcomes.** AKI is often **underdiagnosed** due to vague symptoms ending in being identified too late, increasing **mortality and morbidity rates.**

Solution

Real-time monitoring of creatinine levels that enables faster medical interventions and patient outcomes for AKI through a user-friendly, and non-intrusive device.

Key Metrics

- Patient Outcomes
  - Reduction in AKI incidence
  - Improvement in Recovery Rates
- Hospitalization duration reduction
- Healthcare cost reduction

Unique Value Proposition

The continuous biosensor will provide data for healthcare providers to make informed decisions for **improved patient outcomes** for AKI. Trends and patterns present, that might be missed using periodic testing.
This will also **reduce healthcare costs** by minimizing hospital visits and stays by preventing complications through early detection.

Unfair Advantage

- Powerful intellectual property (IP)
- Strategic collaboration with Med-Tech companies (e.g. Benchmark, Palmsense)
- Unique methods for the identification and evaluation of biomarkers

Channels

- Direct sales: hospitals, clinics, health care facilities
- Online Sales
- Healthcare Conferences and Trade Shows

Customer Segments

- Patients with AKI or who are at risk of developing (ICU patients)
- Healthcare Providers
- Hospitals and Clinics
- Insurance Companies
- Medical Device Companies

Cost Structure

- Research and development (R&D) expenses
- IP costs
- Manufacturing costs
- Expenses for marketing and sales
- Regulatory compliance and certifications costs

Revenue Streams

- Sales for the biosensor device
- Licensing to other medical technology manufacturers
- Data Monetization

Appendix II: Skills and Expertise Needed

The below table shows the minimum predicted personnel needed to meet each of the timeline deadlines which are further described in **Appendix III**. Note that these would be contractors or third parties if for the given task. The core team will involve a head engineer, a biomedical engineer, a computer scientist and a business and marketing persona.

Table 4: Proposed Personnel and Skillset

Activity	Combined Professions/Skillsets Needed	Estimated Number of Individuals
Phase I (2024-2026)		
Establish legal entity BV	Legal expert with business consulting experience	1
Secure initial funding	Financial advisor with grant writing skills	1-2
Establish regulatory framework	Regulatory affairs specialist with quality assurance expertise	1
Phase II (2026-2032)		
Device Optimization and R&D	R&D engineer with software and hardware development skills-> Benchmark and/or PalmSens	2-3
Patent Strategy	Patent attorney with IP consulting experience	1
Network Building and Market Validation	Marketing specialist with business development skills	1-2
Clinical Trials and Regulatory Approvals	Clinical researcher with regulatory affairs expertise	2-3
Phase III (2032-2034)		
Insurances for Business and Risk Management	Insurance broker with risk management consulting skills	1
Device Launch and Sales	Sales manager with marketing and customer support skills	2-3

Funding for growth	Financial advisor with business development skills	1-2

Appendix III: Business Case Timeline Breakdown

Phase I (2024-2026)

- **Activity 1.1:** Establish legal entity **BV (Besloten Venootschap)**
  - **Goal:** Obtain legal structure for the company and will be carried out with support from NovelT.
  - **Duration:** 5 months
- **Activity 1.2**: Secure initial funding through grants, loans, equity investments
  - **Goal**: Provide financial resources for R&D, operations, patenting and clinical trials. This will take place in Seed Round, further details are provided in **Appendix II**
  - **Duration:** 1-2 years
- **Activity 1.3**: Establish initial regulatory framework (identify relevant ISO standards)
  - **Goal**: Lay groundwork for future compliance.
    - Important Standards to take note of and aim for continuously throughout the product lifecycle and update accordingly for compliance
      - **ISO 13485:2016-** Quality management system (QMS)
      - **ISO 14971:2019-** Risk Management
      - **IEC 62366-1:2015-** Application of usability engineering to medical devices
      - **ISO 11135:2014-** Sterilization; In our case we would outsource this to Uneedle
      - **ISO 28219:2017-** Packaging
      - **ISO 20417:2021**- Information supplied by manufacturer
      - **ISO 62304:2006-** Sofware life cycle processes
      - **ISO 14155:2020-** Good clinical practice during trials
      - **ISO 15223-1:2021**- Labelling and information provided by supplier to end-users.
  - **Duration:** 1 year

Phase II (2026-2032)

- **Activity 2.1:** Device Optimization and R&D
  - **Goal:** Using our sponsor Benchmark or PalmSens and Uneedle technology to minimize the device, develop hardware and software for a smart patch system, similar to a non-invasive glucosensor. Create a functional, user-friendly wearable biosensor along with an application for it to store data. This will be outsourced to our sponsor Benchmark or will use PalmSens's services.
  - **Duration:** 2 years
- **Activity 2.2**: Patent Strategy
  - **Goal**: Conduct patentability search, file for patent, and ensure protection of intellectual property. Further details are provided in **Appendix X.**
  - **Duration:** 2 years
- **Activity 2.3**: Network Building and Market Validation
  - **Goal**: Build relationships with healthcare professionals, attend industry events, and gather market insights. Validate device design criteria and refine through interviews with end-users and medical staff.
  - **Duration**: 2 years
- **Activity 2.4**: Clinical Trials and Regulatory Approvals
  - **Goal**: Conduct clinical trials I,II, prepare and submit regulatory submissions (510(k)), and obtain necessary approvals. Apply prior for **Investigational Device Exemption (IDE) in the US** which will allow the device to be used in a clinical study to collect safety and effectiveness data, which can expedite the approval process.
  - **Duration**: 4 years

Phase III (2032-2034)

- **Activity 3.1:** Insurances for Business and Risk Management
  - **Goal:** Establish insurance coverage (goods, inventory, transportation, liability).
  - **Duration**: 3 months
- **Activity 3.2**: Device Launch and Sales
  - **Goal**: Initiate sales and marketing efforts, secure insurance coverage, and implement post-market surveillance
  - **Duration**: 1-2 years

- **Activity 3.3**: Funding for growth
    - **Goal**: Seek additional funding (Series A), explore new markets and establish new partnerships
    - **Duration**: 1 year

Appendix IV: Funding Breakdown

Round 0 Seed Funding:

- Subsidies
    - **OpenMind Grant**- € 50,000 (Open Mind | NWO, n.d.)
    - **Take Off Grant**: (Take-off | NWO, n.d.)
        - Phase 1: Feasibility Study €20,000-40,000. Leads to a report which includes the possibilities of a start-up. (max 6months)
        - Phase 2: Early Stage Route. € 50,000-250,000 (max 24 months)
    - **SME Innovation simulation region and top sectors**
        - Early Stage Financing (EFF): Total budget € 1,750,000 (Vroegefasefinanciering (VFF), n.d.)
        - MIT scheme (Mkb-Innovatiestimulering Regio En Topsectoren (MIT) - Topsector ICT, n.d.)
        - MIT: R&D Collaborative Projects: Total budget for Overijssel region €2,240,000
        - MIT Knowledge Vouchers: Max large voucher value €9,000; Max small voucher value €5,250.
        - MIT: TKI Innovation Brokers; €10,000
        - MIT: TKI Network Activities; €1,000
    - **Angel Investor Network**: (Business Angels Oost NL | Oost NL, n.d.)
        - €50,000 and €250,000 average
    - **Demonstrator Grant** (Demonstrator | NWO, n.d.)
        - Aims for startup to reach TRL 4 of device „technology validated in lab“
        - Total available 1 million; max per project is 160,000#
    - **Innovation University of Twente vouchers** (UT Innovation Vouchers, n.d.)
        - Total 50 vouchers, each worth € 10,000
- Loans
    - **NovelT Top Loan**
        - €40,000, with an interest rate of 7.75%; Standard duration of 4 years;
        - First 2 years are interest-only;
    - **CrowdFunding** (Crowdfunding Platform Geldvoorelkaar Sinds 2011 in Nederland, n.d.)
        - Amount is for us to decide; 6-10% interest rate for duration of 5 years
    - **Dutch Student Investment Fund** (Home - DSIF, n.d.)
        - €5.000 to €50.000
- Equity
    - **Oost nl** (Fondsen | Oost NL, n.d.)
        - Startup Fund Gelderland (SFG); € 30,000-100,000
        - ION+; € 200,000-2,500,000. (for technological development in the riskiest phase)
    - **Cottonwood Technology Fund oost** (Early Stage | Deeptech VC: Cottonwood Technology Fund, n.d.)
        - 1M – 3M for seed round
- Venture Capital
    - **Innovation Industries** (Innovation Industries | European Impact and Deep Tech VC, n.d.)
        - € 250k-50,000k
    - **Health Innovations** (Health Innovations, n.d.)
        - € <150k
- Other
    - **High Tech Lease Fund** (High Tech Fund - Kennispark, n.d.)
        - Non-profit lease facility for manufacturing equipment for startups
    - **MedTech Lease Fund** (High Tech Funds, n.d.)
        - Same as the previous but with a focus on Medical Technical Equipment
    - **Innovation Box** (Innovatiebox, n.d.)
        - Provides tax benefits for profits coming from innovative activities. Lower tax rate for startups- 9%
    - **Kickstarter Campaign** (Kickstarter, n.d.)
        - Pure Crowdfunding

Round A Seed Funding (start phase):

- Subsidies
    - **SME Loan Guarantee (BMKB)** (Borgstelling MKB-Kredieten (BMKB), n.d.)
        - Max at 1.5 million
    - **EFRO** (Welkom Bij OP Oost, n.d.)
        - REACT-EU- First Tranche Total available €5.9 million. Second Trance Total available €50 million. (REACT-EU, n.d.)
        - ION +2- Total available sum of €5.5 million (ION+2, n.d.)
        - ION+- Total available sum of €7.5 million (ION+, n.d.)
    - **Horizon Europe** (Horizon Europe | Onderzoek En Innovatie, n.d.)
        - €95.5 billion spanning from 2021 to 2027
    - **Eurostars**: subsidy for international market-oriented R&D (Eurostars: Subsidie Internationale Marktgerichte R&D, n.d.)
        - €22 million available annually for the Eurostars programme
    - **DHI subsidy scheme** (DHI-Subsidieregeling, n.d.)
        - Max of €100,000
- Equity, Venture Capital, Others
    - Same as previous Seed Round still apply

Appendix V: Key Partners Relationship

As a student team, we offer brand awareness, a pool of talent for the relevant company for networking or future internships. We also can pitch to classes about the potential sponsor or partnering company. In return we would get monetization and knowledge transfer. For investors we would negotiate in a clear agreement the responsibilities, revenue sharing, IP ownership and company equity.

Table 5: Key Partner Rubric

Partner Type	Key Partner	What they provide	What we offer
Research Collaborators	Molecular NanoFabrication, BioElectronics, RaM, BIOS	Specialized facilities, expertise in microfluidics, surface chemistry	Access to our biosensor technology, potential for joint research, student internships
Manufacturing Partners	Benchmark, PalmSens, Uneedle	Manufacturing capabilities, device miniaturization, software development	Potential for licensing our technology, joint product development
Business Support	NovelT, Incubase	Legal, financial, and startup support, networking opportunities	Access to our innovative technology, potential for equity investment
Funding Sources	TechMed Centre, Design Lab, Benchmark, Oost nl, ION+, Cottonwood Technology Fund, Angel Investors, Government Grants (OpenMind, Take Off, SME Loan Guarantee, EFRO, Horizon Europe, Eurostars, DHI)	Financial resources, mentorship, industry connections	Equity, intellectual property rights, potential for high returns
Regulatory Bodies	FDA, CE Marking authorities	Regulatory guidelines, approval processes	Compliance with regulations, market access
Healthcare Providers	Hospitals, Clinics, Medical Practitioners	Clinical testing sites, patient feedback, potential early adopters	Access to real-world data, potential for collaborative research
Industry Associations	Renal Healthcare Associations, Medical Device Industry Associations	Industry insights, networking opportunities, policy advocacy	Membership fees, participation in industry initiatives

Appendix VI: Market Share, Financial Timeline Breakdown and Assumptions

Market Share Calculation

Research on the global biosensor market size suggests it is approximately 27.5 billion euros. According to the same source, the USA dominates 40% of this market, accounting for 11 billion euros. Europe contributes around 24%, equivalent to 6.6 billion euros. Electrochemical sensors generate 70% of the total revenue, and from those, home healthcare diagnostics make up 20%. (Biosensors Market Size to Worth Around USD 58.44 Bn by 2033, n.d.)

Finally, the electrochemical biosensor market share for home healthcare diagnostics for the US comes to about 1.54 billion euros. Assuming our device can detect and diagnose 10% of the various biomarkers associated with different diseases after finalizing research and development, a more realistic value would be 154 million euros.

The adoption of EU market would bring an additional 50% to the 154 million from the US market share size- totaling at 246 million euros. (Biosensors Market Size to Worth Around USD 58.44 Bn by 2033, n.d.)

An important assumption is that only the regions with largest share are accounted for the above data source, out of entire continents. For a figure with more precision further analysis and access to data in these domains is required and should be explored.

Financial Timeline Breakdown and Assumptions

		Phase I: Foundation		Phase II: Development & Validation						Phase III: Market	
	Year	1	2	3	4	5	6	7	8	9	10
Revenue Stream											
Standard subscriptionmodel	Price per unit (€)	669.5	669.5	669.5	669.5	669.5	669.5	669.5	669.5	669.5	669.5
	Number of units	0	0	2	5	10	15	22	30	60	150
	Total sold units	0	0	0	0	0	3	5	8	20	30
	Revenue	0	0	0	0	0	2008.5	3347.5	5356	13390	20085
Premium subscription model	Price per unit (€)	717.5	717.5	717.5	717.5	717.5	717.5	717.5	717.5	717.5	717.5
	Number of units	0	0	0	5	10	15	22	30	60	100
	Total sold units	0	0	0	0	0	2	3	5	12	25
	Revenue	0	0	0	0	0	1435	2152.5	3587.5	8610	17937.5
Smart Patch System	Price per unit (€)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
	Number of units	0	0	5	20	50	60	80	120	210	320
	Total sold units	0	0	0	0	0	45	72	117	288	495
	Revenue	0	0	0	0	0	9450	20160	49140	211680	554400
Data Monetization	Total Data points	0	0	3	10	15	22	35	70	150	300
	Customers	0	0	0	0	0	2	3	5	7	12
	Cost per data point/case	15	15	15	15	15	15	15	15	15	15
	Revenue	0	0	0	0	0	660	1575	5250	15750	54000
Licencing	Price per unit (€)	20000	20000	20000	20000	20000	20000	20000	30000	35000	40000
	Customers	0	0	0	0	1	1	2	4	5	5
	Revenue	0	0	0	0	20000	20000	40000	120000	175000	200000
Total Generated Revenue Streams(€)		0	0	0	0	20000	33553.5	67235	183333.5	424430	846422.5
Personnel Salary (prior to deductions)											
Team Lead (Engineer,PhD)	Sallary(€)	95000	95000	96900	98838	102791.5	106903.2	111179.3	115626.5	120251.5	125061.6
Biomedical Engineer (PhD)	Sallary(€)	75000	75000	76500	78030	81151.2	84397.2	87773.1	91284.1	94935.4	98732.8
Software Engineer/Computer Scientist (MSc)	Sallary(€)	77000	77000	78540	80110.8	83315.2	86647.8	90113.8	93718.3	97467	101365.7
Legal Advisor	Sallary(€)	-	-	-	-	79000	81370	83811.1	86325.43	89778.5	93369.6
Sales and Marketing	Sallary(€)	-	-	-	-	-	-	60000	61800	64272	66842.88
Total Salary Expense (-€)		247000	247000	251940	256978.8	346258	359318.3	432877.3	448754.3	466704.5	485372.6
Funding (€)											
OpenMind Grant		50000	0	0	0	0	0	0	0	0	0
	Phase I Feasibility Study	20000	0	0	0	0	0	0	0	0	0
Take-off Grant	Phasell Early Stage Route	0	50000	0	0	0	0	0	0	0	0
	MIT Knowledge Vouchers	14250	0	0	0	0	0	0	0	0	0
	MIT R&D Collab Projects	67200	0				0	0	0	0	0
SME Innovation	Innovation Brokers	10000	0	0	0	0	0	0	0	0	0
	TKI Network Activities	0	1000	0	0	0	0	0	0	0	0
Demonstrator Grant		0	80000	0	0	0	0	0	0	0	0
UT Innovation Vouchers		10000	0	0	0	0	0	0	0	0	0
NovelT Top Loan		0	0	40,000	0	0	0	0	0	0	0
KickStarter Campaign		0	0	0	0	0	0	0	0	0	0
	ION+	0	0	0	0	200000	0	0	0	0	0
Venture Capital	Health Innovations	0	0	0	0	150000	0	0	0	0	0
	Cottonwood Technology Fund oost	1500000	0	0	0	0	0	0	0	0	0
Eurostar Subsidy		0	0	0	0	0	0	0	0	200000	0
DHI Subsidy Scheme		0	0	0	0	0	0	0	0	50000	0
Total Funding Secured (€)		1671450	131000	40000	0	350000	0	0	0	250000	0
Other Costs (€)											
IP		10800	40040	70080	4140	4160	4200	4280	4320	4360	4400
Marketing		0	0	0	0	10000	0	0	30000	10000	10000
Operational costs (Delivery and Packaging)		0	0	0	0	0	195	312	507	1248	2145
Total Other Costs (-€)		10800	40040	70080	4140	14160	4395	4592	34827	15608	16545
Net Income (€)		1413650	-156040	-282020	-261119	9582.048	-330160	-370234	-300248	192117.5	344504.9
Cumulative Wealth (€)		1413650	1257610	975590	714471.2	724053.2	393893.5	23659.18	-276589	-84471.1	260033.8

Figure 11: Excel Worksheet used to formulate the 10-year financial timeline

Assumptions used include:

-Inflation was not accounted during the next 10 years, as such it is likely that costs of materials would increase, hence driving us to match this in terms of pricing of the device and goods offered.

-Only some of the Funding opportunities are listed from the total list provided in Appendix IV. It is likely that more options will be targeted to secure higher funding, again with the focus being on subsidies and government funds, relevant to the MedTech innovation sector.
-During the Research and Development phase II the targeted research and hospital institutions will be in the local area of our university. Potential interested parties would be MST hospital, UMC Utrecht, Erasmus MC, and university research groups with a focus on renal diseases. Hence why a modest number was adopted. An increased number is observed towards the end of the Phase II as plans will be made to slowly introduce to the US and familiarize with the ecosystem there. Focusing first research institutions in similar fields. Once FDA approval is granted, targeted institutions will include hospitals with a high capacity for ICUs and that are renowned for invasive surgeries- John's Hopkins Hospital, Mayo Clinic, Cleveland Clinic, among others.
-Marketing focus is placed in later years before market launch to build momentum, with more moderate costs once launch is announced. Numbers are subject to change depending on chosen marketing strategy and targeted platforms.

Appendix VII: Device Costs

Table 6: Sensor Material Costs per device

Material/Item	Price (€)
Power Bank	35
LCD Screen	74.3
3D Printed Device Parts	16
Labour- Assembly (25€/h, 3h)	75
Labour- PDMS Casting and Bonding (25€/h, 2h)	50
Raspberry Pi 4 Inbuilt Computer	53
Total	**303.3**

Table 7: Chip Material Costs per device

Material/Item	**Price (€)**
ABS Filament (710g)	14.2
Labour – Functionalizing, Quality Control (25€/h)	75
Solvents: MilliQ, PBS	0.8
Cleaning: Acetone, IPA, Toluene, Ammonia	1.4
Sylgard PDMS	0.4
3D Printed Chip Holder	0.3
Interdigitated Electrodes	22.4
SQ-MODOL-24038 3' Methylene Blue (Atto MB2) via Thiol C6 scale 1000 nmol	6.7
Total	**121.2**

Table 8:Assembly and Shipment Costs per device

Material/Item	**Price (€)**
Assembly (25€/h)	37.5
Packaging	0.25
Delivery	1
Transportation Insurance	0.25
Total	**39**

Appendix VIII: Design Iterations

Design ideations and sketches:

It was initially decided to add in the set-up a control chip in series with the sensing chip, both with the same fabrication, cleaning and functionalization processes. However the control chip would have been functionalized with scrambled aptamers, which would not bind to any component in the serum. The idea behind having a control chip inside of the device was in order to reduce the noise in the signal, and make sure of the specificity of the binding. This idea was however not brought forward in the development, due to time constraints.

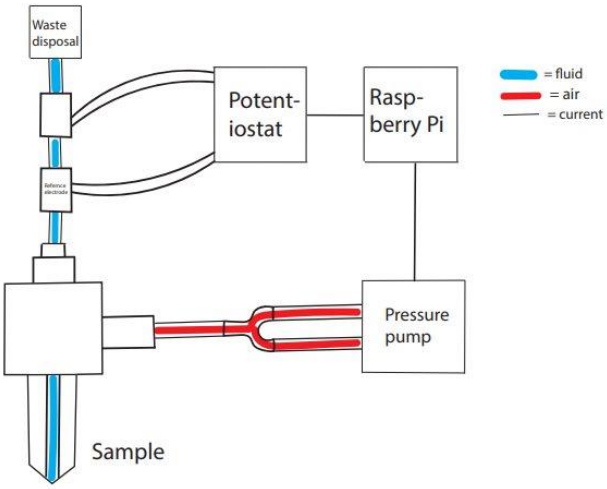

Figure 12. Original set-up schematic

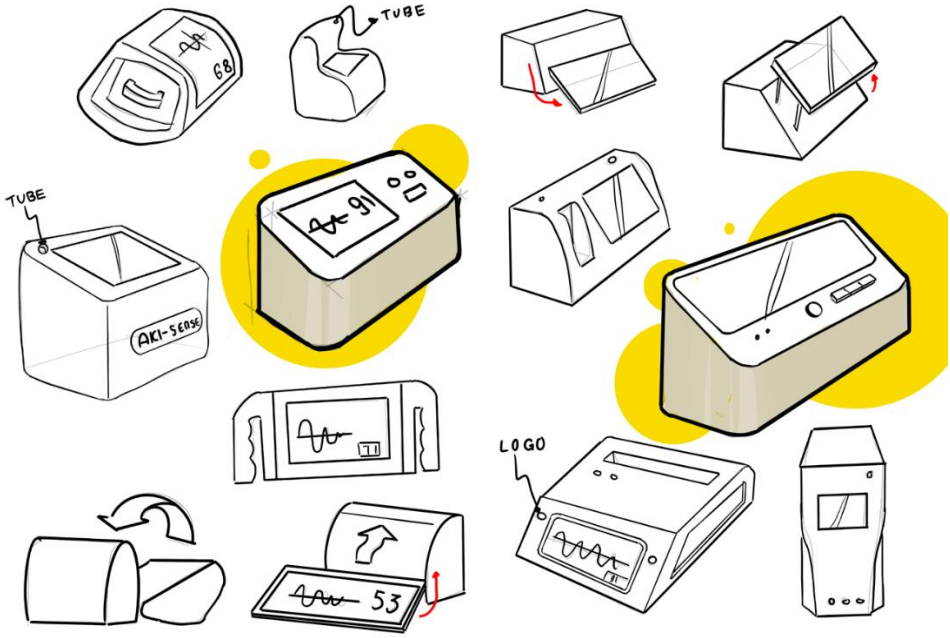

Figure 13. Sketches during the designing process

Final Design:

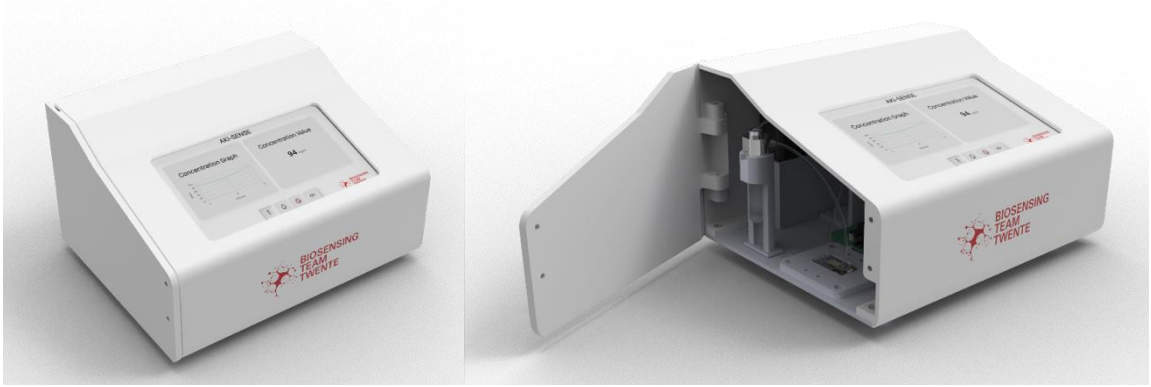

Figure 14. Outer view of the device

Appendix IX: Alternative functionalization

In the QCM-D experiments, an antifouling layer with PLL, MCH and bare gold were assessed in which PLL and MCH were flowed over two separate chips, followed by human serum. The QCM-D results indicated that both PLL and MCH exhibited similar antifouling properties, with PLL presenting a slightly better antifouling performance compared to MCH. The latter can be seen due to the smaller decrease in frequency in **Error! Reference source not found.**. These results indicate that both components can be used for functionalization.

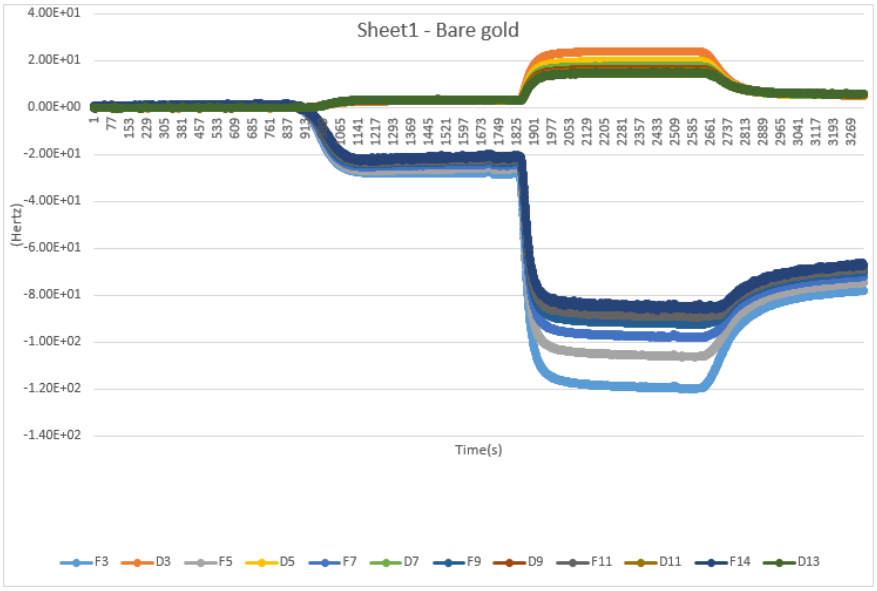

Figure 15. QCM-D result for bare gold

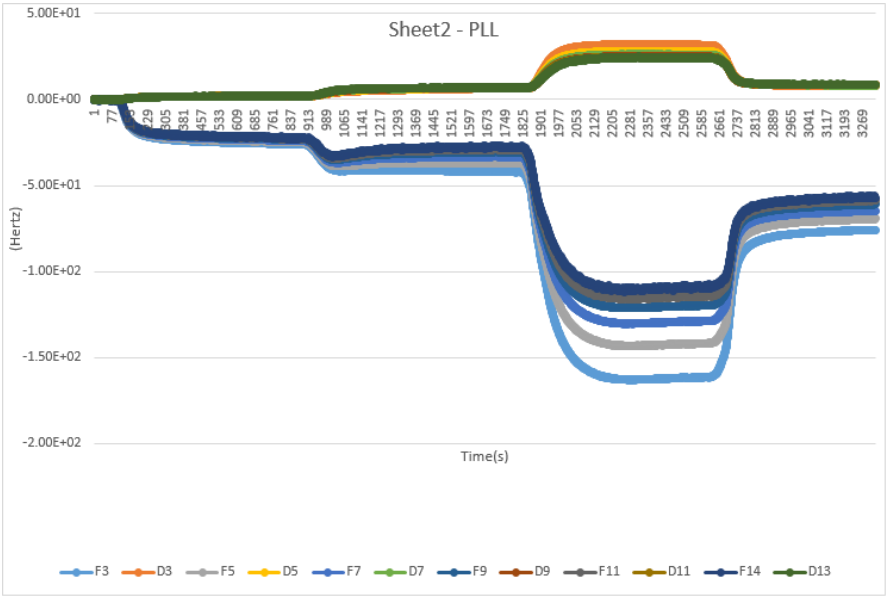

Figure 16. QCM-D result for PLL functionalization

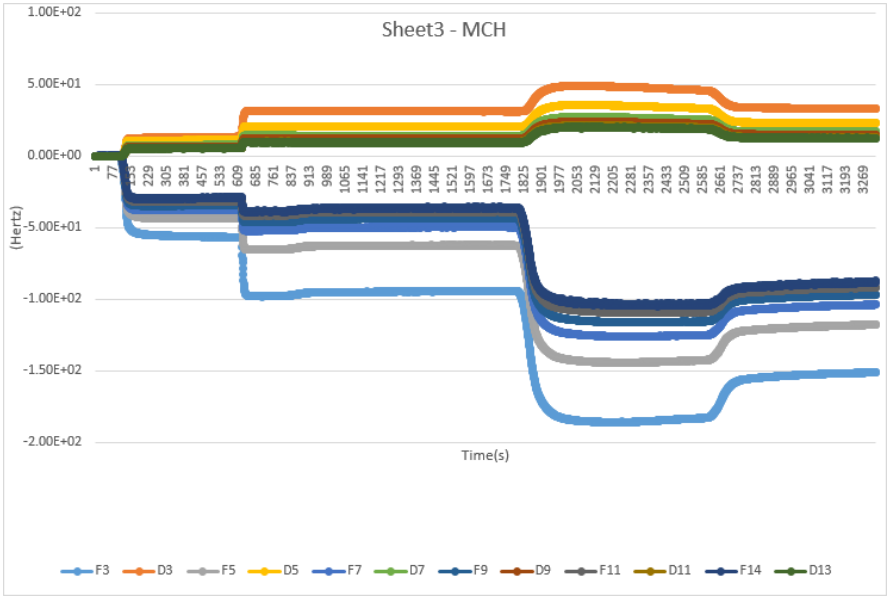

Figure 17. QCM-D result for MCH functionalization

Nevertheless, due to time constraints, MCH was first selected as an antifouling layer. As previously mentioned, this layer was later discarded due to the obstruction of the binding signal.

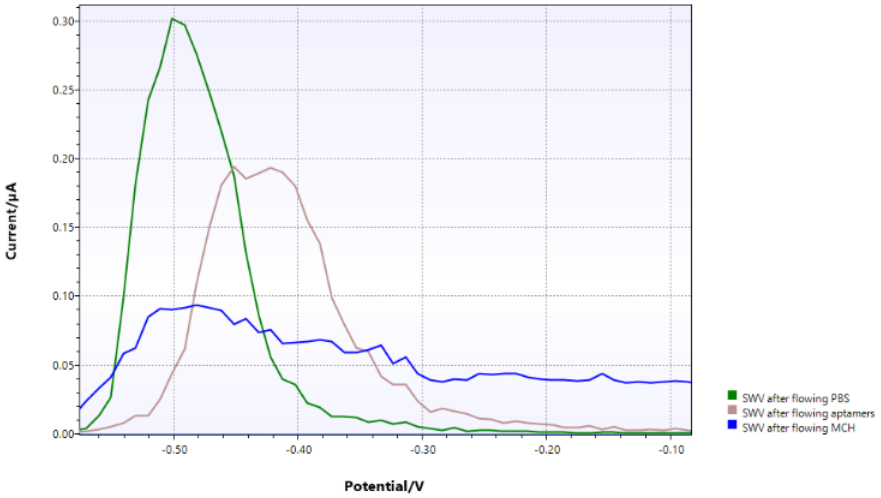

Figure 18. SWV results from bare gold (green), gold after flowing aptamers (brown) and after flowing MCH (blue).

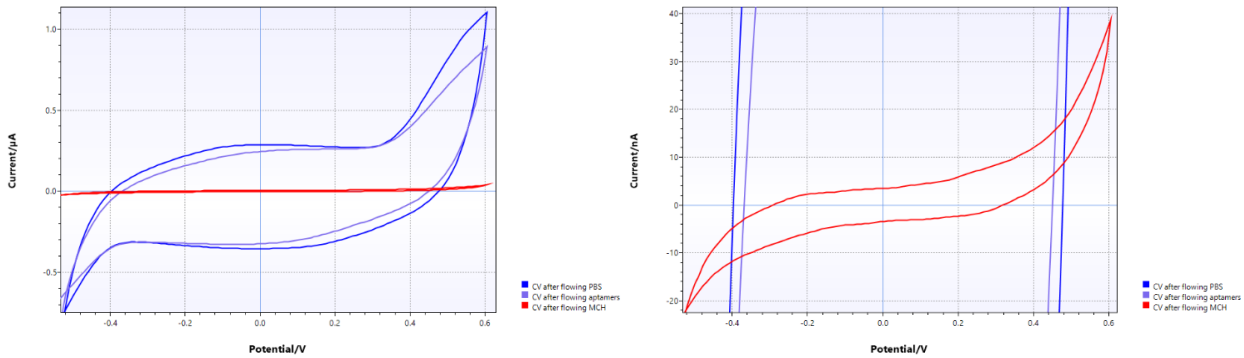

Figure 19. CV results from bare gold (dark blue), gold after flowing aptamers (light blue) and after flowing MCH (red). a) complete graph, on the left; b) zoom on CV after MCH, on the right.

In Figure 18 and Figure 19, it can be seen that the measurements for the MCH flow have inaccurate results. In the graph for the SWV measurements, it is observed that the measurement for the MCH fades away the peak of the previous measurement. The same is observed for the CV measurements, where the results is much smaller than the previous measurements. These results, in addition to no change in the SWV when flowing samples, lead to conclude that the MCH would not allow any signal to be observed irrespective of what would happen on the electrode surface.

Appendix X: Starting Capital Criteria Breakdown

Table 9: Summary Table with Key terms

Category	Key Terms
R&D Expenses	Personnel Costs, Equipment & Materials, Facilities Rent, 3rd Party Expertise, Training
Regulatory Compliance	FDA Clearance, Clinical Trials, Consultations, Compliance with Current Good Manufacturing Practice (CGMP), Data Integrity
Data Protection	Health Insurance Portability and Accountability Act (HIPAA), Federal Information Security Management Act (FISMA), Data Security Measures
IP Protection	Patentability Search, Freedom to Operate (FTO) Analysis, Patent Application, Legal Fees, Patent Cooperation Treaty (PCT), Patent Office Consultations
Manufacturing Setup	Machinery Acquisition, Facility Costs, Raw Materials, Quality Assurance, Waste Management
Marketing & Distribution	Branding, Advertising, Digital Footprint, Distribution Channels, Trade Shows, Personnel Costs

R&D Expenses-Involve costs for the research and development of the technology and methods used for the creatinine biosensor and continuous improvement progress. These include researchers, technicians and staff salaries and relevant training qualifications, equipment, materials and consumables used for experiment trials and analysis, 3rd party expertise and consultations and facilities rent to be used for work and storage. The end goal being developing a smart-patch device that can be controlled through an application.

Regulatory Compliance- Involve costs linked to regulatory bodies that are needed for ensuring compliance and certification of the product to allow it to the relevant market. In our case this would break up into consultations from 3rd party contractors who specialise in regulatory affairs to check the documentation and forms that need to be completed. The different geographic regions would have their own regulations such as the FDA for the USA and and CE mark for the European Economic Area (EAA).

A 501(k)-clearance form might be required after expertise guidance if our device is similar to an already existing one on the market. We believe the end version might require one due to similarities to a gluco-sensor and patch system on the outlook of the device.

It is important to note that prior to getting regulatory compliance, FDA, for the device, clinical trials have to be carried out to validate the function and safety of the biosensor. These require adherence to guidelines/regulations, funding and time. It is important to note to follow Good Clinical Practice (GCP) guidelines and International Investigator Statement (IIS) so that the findings from the trials done in the US can be accepted when applying for other regulatory approval such as the CE mark in Europe (New Statement To Replace The FDA 1572 Form For Non-IND Trials Supported By NCI - EORTC, n.d.; Rules for Clinical Trials | Medicines | Health and Youth Care Inspectorate, n.d.).

CE certification would require the intervention of a notified body as our biosensor would fall within the class IIb category (Notified Bodies - European Commission, n.d.). The approved notified bodies within the Netherlands are BSI Group The Netherlands B.V., DEKRA Certification B.V. and Kiwa Dare B.V. (Role of the Notified Body | Medical Technology | Health and Youth Care Inspectorate, n.d.). We would in turn need to register the device in the EUDAMED data base and follow up with a clinical evaluation to prove the safety and usability of the biosensor. Each step has fees and costs that need to be considered. It is important to hire expertise consultants for this conformity assessment as it directly impacts the market accessibility timeline and places a higher financial burden to keep the project afloat if reiterations are needed.

In addition, because the device will store patient data and transfer it to the relevant medical institution, certain measures for data safety must be in place. The General Data Protection Regulation (GDPR) must be adhered to along with security measures so that the information cannot be easily breached for the EU (Data Protection under GDPR - Your Europe, n.d.). Similarly, for the US, the Health Insurance Portability and Accountability Act (HIPAA) and Federal Information Security Management Act (FISMA) need to be complied to (EU vs US: What Are the Differences Between Their Data Privacy Laws? | Endpoint Protector, n.d.). These too will incur additional expertise and consultancy costs.

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adhered to along with security measures so that the information cannot be easily breached for the EU (Data Protection under GDPR - Your Europe, n.d.).These too will incur additional expertise and consultancy costs.
Cost breakdown:

- **Quality Management System (QMS) Compliance**:
    - Identify and analyze areas in QMS to comply with FDA regulation: ~**€25,000**
- **Medical Device Regulation Certification**:
    - Apply for medical device DFA clearance: **+€100,000**
- **Conformity Assessment for Class II (FDA)**:
    - Cost of conformity assessment: ~**€15,000**
- **Post-Market Surveillance**:
    - Applicable for Section 522 of the Federal Food, Drug, and Cosmetic Act (FD&C) and FDA: ~**€20,000**
- **Training and Education on Distribution Requirements**:
    - Training and education plan requirement by EU on EUMDR distribution requirements: **Up to €20,000**
- **Clinical Trials**:
    - Clinical trials according to Regulation EU No 536/2014: **€50,000 to €500,000 (time-dependent)** (Clinical Trials - Regulation EU No 536/2014 - European Commission, n.d.)
- **510(k):**
    - FDA Medical Device Registration Fee (Small Business Fee), takes up to 40 months (How Much Does It Cost to Develop a Medical Device? FOUR METHODS TO ESTIMATE MEDICAL DEVICE AND POINT OF CARE DIAGNOSTIC DEVELOPMENT COSTS, n.d.; Medical Device User Fee Amendments (MDUFA) | FDA, n.d.): **€ 7,077**

Intellectual Property (IP) Protection- Prior to filing a patent it is important to carry out a patentability search to assess the product's suitability to be patented. It would cost €800 (How Can I Apply for a Patent? | Government.NI, n.d.). Following this, a **Freedom to Operate (FTO) Analysis** would assess if our technology would infringe on someone else's intellectual property (IP). This can be handled by advisors from the **Netherlands Patent Office (OCNL**). Such a risk analysis is beneficial also in the eyes of potential investors who prefer to minimize their exposure to risk (Patent Blog: Convince Investors with Your Freedom to Operate (FTO) Analysis, n.d.).
Ones in the Netherlands can apply for a maximum of **20 years and take up to 18 months** to get processed. The stages of the process are as follows**: invention disclosure document, prior art search, patent application, patent examination, patent issuance, enforcement**. It is important to consider hiring **a patent attorney** to avoid pitfalls such as patent infringement, trade secret protection, competition and to aid in the complexity of patentability (Intellectual Property Strategies For Medical Devices, n.d.). Overall an application in the Netherlands costs between €2,000 and €10,000. Maintenance fees increase with each ear starting from €40 to €1,400. Enforcement costs are estimated to be between €20,000 and €70,000.

For our expansion into other markets the patent will need to follow the **Patent Cooperation Treaty (or PCT)** to gain international accreditation. For a fee of € 4,000 to € 5,000 a year the local Dutch one can be transformed according to whichever countries are targeted. Entire PCT procedure (application, European and national phase and costs for a patent attorney) would be between € 50,000 to € 100,000 (Filing a Patent Application Worldwide, n.d.).

Table 10: Intellectual Property Breakdown

IP Protection Process	Costs and Duration
Patent Application (NL)	€10,000 (18 months processing)
Maintenance (NL)	€40 - €1,400/year (max 20 years)
Enforcement (NL)	~€50,000
PCT International Application	~€75,000 (including attorney fees)

Manufacturing Setup-
This accounts for the machinery needed to manufacture the components of the biosensor and to assemble it. A further breakdown involves costs for purchasing or leasing the machinery needed; facility setup, rent and the utility

costs; raw materials and components costs; quality assurance and maintenance of machinery; scaling up as the expansion to new geographic regions; waste management for disposal and recycling. An important factor is having a contingency fund for when unforeseen expenses may arise. This reserve budget would allow for immediate reaction with minimal loss in the process.

Marketing and Distribution-
Involves costs linked with the marketing strategy in place which aims to promote the branding of the device, raise awareness of the current healthcare problem, and utilize multiple distribution channels (such as LinkedIn and Instagram). By utilizing effective means, the biosensor would gain popularity and momentum to aid in its spread across the targeted markets. Branding would involve our company identity, logo, packaging, and materials used for promotion such as flyers and stickers. Advertisements would also be vital in our mission which do have a cost, depending on the chosen medium. Building a digital footprint through social media will allow the device to reach a wide audience. The distribution channels would be based on partnerships with distributors which may involve monetary values. Pure logistics would account for storage, handling, and transportation of the biosensor to the relevant medical institution or retailer. An effort will be made to attend trade shows and conferences linked to biosensors, to Nephrology and other related fields, which all will have participation fees. Sales and marketing personnel would be in charge of this area mainly. Their salaries and commuting costs are also to be accounted for.

Appendix XI: Interviews with medical and other professionals

The following section provides information on the contacts made for the business case development.
Table 11: Summary Contacts Table

Name	Profession	Theme
PhD Fokko Wierenga	Researcher and Innovator	Medical Biosensors
Nikki van de Veerdonk	Renal Program Coordinator	Early Disease Detection
PhD Dimitrios Stamatialis	Renal Researcher	Kidney Health
Dr Goos Laverman	Product Development Specialist	At home monitoring of AKI
Dr Christina Gant	Healthcare Strategist	
PhD Dion Martines	Medical Device Designer	
MD Lluís Guirado	Medical Doctor – Nephrologist	AKI Detection
MD Roger Alabau Perich	Medical Doctor - Nephrologist	Nephrology
Tim Jungman	Business Analyst	Market Strategy
Niels Jansen	Patent Advisor	Intellectual Property
Jeroen Wissink	Healthcare Entrepreneur	MedTech Innovation
Dr Marcel Zevenbergen	Research Scientist	Patient, Practitioner, Insurance Criteria

Interview with Prof. Dr. Goos Laverman, PhD Christina Gant, PhD candidate Dion de Martines

- Project Focus:
- Interest in at-home monitoring of high-risk patients for early AKI detection.

- Potential Patient Groups for Monitoring:
- Patients with chronic kidney disease receiving medication, at risk during incidents like diarrhea.
- Those who received donor kidneys and are discharged post-operation.
- Patients undergoing medication changes, especially after kidney transplant or for heart failure, diabetes, or obesity.

- Regulatory and Reimbursement Challenges:
- Need to demonstrate added value over current practices to justify reimbursement.
- Selection of high-risk patients crucial for cost-effectiveness.
- Concerns about false positives, reliability, and fluctuations in creatinine levels throughout the day.
- Legislation considerations on data transmission and responsibility for diagnosis.
- Complexity of AKI research endpoints makes proving device efficacy challenging.

- Recommendations:
- Focus on understanding daily creatinine variations for AKI diagnosis before considering preventive measures.
- Establish clear boundaries and objectives for the project to facilitate progress.

Interview with MD Lluís Guirado (Nephrology director Fundació Puigvert)

What are the current methods and biomarkers/parameters considered most important for the diagnosis of AKI? In our case the most important is the plasma creatinine and derived from it the glomerular filtrate extrapolated by the CKD-EPI formula. Together we use plasma and urine urea, urine creatinine, urine Na pl, proteinuria, microalbuminuria and microhematuria (dysmorphic or not). All of them help us define the etiology of ARI.
What problems do you currently encounter in the process of diagnosing and monitoring ARI? That creatinine pl is not the gold standard but is the most adequate in the balance between accessibility and accuracy.

How long does it take from testing to receiving a lab result for ARI?
Half an hour if we ask for it urgently
It depends.
If we talk about analysis time:
a) in a POCT system (Point of care testing) the result can be obtained in < 10 minutes.
b) in a conventional system (central laboratory) results can be obtained in 50 minutes or less (taking into account centrifugation and analysis times).
If we take into account the pre-analytical phase (the doctor makes the request, the nurse pricks it, the sample arrives at the laboratory.....) the time increases considerably (up to 2 hours, easily or even more)

Interview with MD Roger Alabau Perich

As for creatinine, the speed of detection is fast and cheap and accurate, except for some cases of patients with special characteristics that make creatinine not completely reliable and cystatin is used.
Continuous monitoring is not of much use. If possible in critics who need intensive monitoring but for now there would be no benefit.

Interview with MD Arnau Solà

After an operation if it is not well controlled and becomes dehydrated or has heavy bleeding, it can cause AKI, pre-renal when you lose a lot of volume because you are dehydrated or lose a 3rd space, such as someone who has ascites, a cis-rotic or a burned or someone who loses a lot of blood after an operation, the kidney doesn't get enough blood and starts to suffocate. Then you have the parenchymatous ones, with problems of the kidney tissue itself because you give it an antibiotic with too high a titre and it is toxic for the kidney cells or because you put an iodine counter because you want to do a scan to look at the vascular structures and that sucks the kidney a lot, it depends on the type of operation, if they stir the vessels around the kidney or with a risk of bleeding it makes sense to look at the AKI, it must also be said that it is quite a clinical thing, it is mainly measured with the diuresis, if you start urinating very little they suspect acute kidney failure and start looking at all these things.