Team Results Document

AgroSens

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SensUs

SensUs 2024
Acute Kidney Injury

Abstract

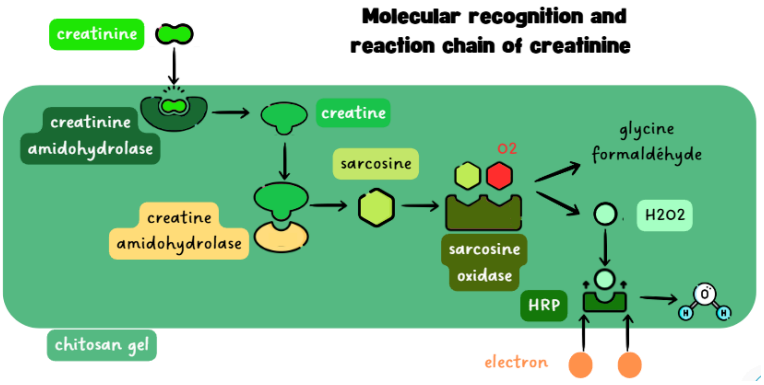
We have developed an amperometric electrochemical biosensor for efficient and precise creatinine detection, leveraging a multi-enzyme system immobilized within a chitosan gel crosslinked with glutaraldehyde. To mitigate interference from creatine and other electrochemically active species, we incorporated a Nafion layer and designed a microfluidic circuit for accurate noise subtraction from the final signal and enabling continuous detection of creatinine.

The ultimate goal of this biosensor as a competitive product on the market is to serve as a versatile platform capable of continuous monitoring of various analytes beyond creatinine. By integrating advanced data analytics, including machine learning, this platform would open new opportunities for precoce diagnosis and management of renal insufficiency, offering insights into disease progression and intervention strategies.

Biosensor

1. Molecular Recognition

Our biosensor utilizes enzyme catalysis for its high specificity, efficiency, and biocompatibility, which ensure accurate and reliable detection of the target analyte. They also have a high turn-over capacity, making them good candidates for continuous sensing. The sensing zone features a chitosan gel matrix[5,6] fixed by electrodeposition[7] to have a stable layer on the gold electrode embedded with four enzymes: creatinine amidohydrolase, creatine amidinohydrolase, sarcosine oxidase, and peroxidase.[1,2] Glutaraldehyde was used for enzyme immobilization.[3] Creatinine is first hydrolyzed by creatinine amidohydrolase to form creatine, which is then converted into sarcosine by creatine amidinohydrolase. Sarcosine is oxidized by sarcosine oxidase, producing glycine, formaldehyde, and hydrogen peroxide (H_2O_2). Finally, peroxidase reduces hydrogen peroxide, completing the reaction sequence.



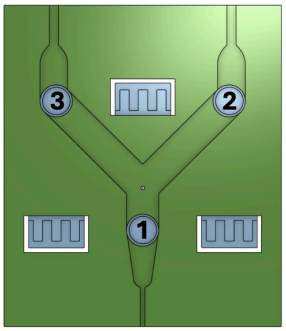
2. Physical transduction

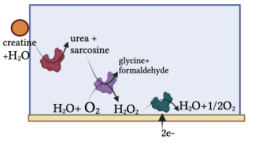
The enzymatic reactions in our biosensor generate an electrical signal by oxidizing hydrogen peroxide at the electrode surface. This oxidation consumes electrons, producing a measurable current that directly correlates with the creatinine concentration. Amperometry, used for transduction, applies a constant potential to the electrode to favor hydrogen peroxide oxidation. As hydrogen peroxide is oxidized, it releases electrons to the electrode, creating a current. This current is proportional to the hydrogen peroxide concentration, and thus, to the creatinine level in the sample. Consequently, the measured current provides a quantifiable signal for precise creatinine detection.

3. Cartridge technology

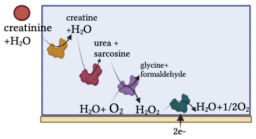
To minimize sensor interference from electrochemically active species and creatine, we implemented three strategies: **Preemptive Creatine Degradation**—the sample passes through a gel that degrades creatine, preserving enzyme activity; **Parallel Measurement**—creatinine concentration is measured after the creatine-degrading gel and subtracted from the total signal to isolate creatinine, with data science techniques applied to reduce noise; and **Nafion Layer Application**—a Nafion layer prevents

contamination by electrochemically active species, ensuring specificity to the target analyte. Microfluidics were employed to handle low sample volumes, facilitate the three required measurements and especially to allow the continuous measure.[4]





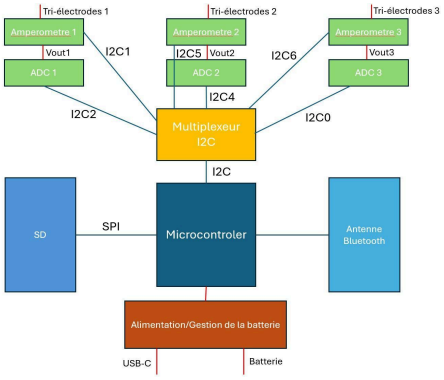
Sensing area 1 & 2: creatine detection and degradation

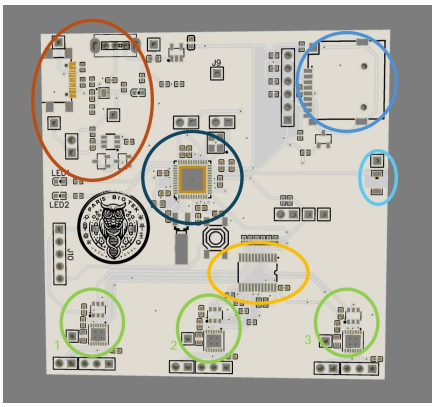


Sensing area 3: creatinine detection

4. Reader instrument

The electronics part of the biosensor consists of 3 amperometric channels connected to the 3 tri-electrodes. These channels are linked to a bluetooth microcontroller with an SD card.

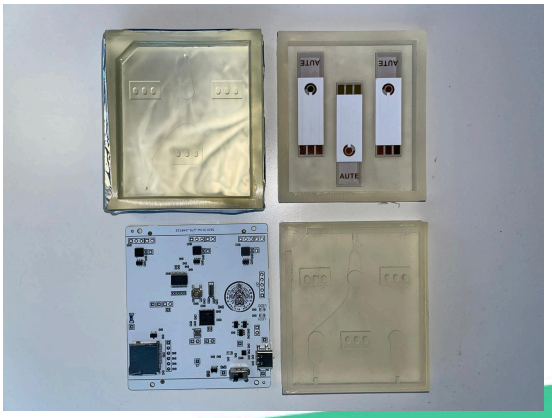




These components were implemented with deployability in mind. To take it into account, the card needed to be small and cheap to produce, with a long autonomy.

5. User interaction

Once the sensor is plugged, the board is ready to be used. Measures are automatically stored on the SD card and later transferred by bluetooth for monitoring on the dedicated mobile application. The sensor works on a battery which can be charged via usb-c. In the future, the sensor will also check other vital signs like the blood level of glucose or proteins. The app could compile the data and send to the patient's doctor each week's results. That way, the doctor can adapt any treatments as needed.



Technical Feasibility

1. Molecular Recognition

We used spectrophotometry, testing various concentrations and temperatures with creatinase, creatininase or both. It was a simple way to ensure that our enzymes were functioning correctly in the gel as the DAB, when in contact with H2O2, produces a brown deposit.

We tested the enzymes at both 37°C and 50°C, with 50°C showing significantly better results. With both creatinase and creatininase with a concentration of 1U/mL in a gel, we were able to detect the presence of 0.1 M of creatinine, which was satisfying considering the method used.

However, this technique lacks the precision needed and would be difficult to scale down for use in an embedded biosensor. Therefore, we turned to amperometry, which, according to the literature, better aligns with our expectations. We are currently in the process of experimenting with this method.

2. Physical transduction

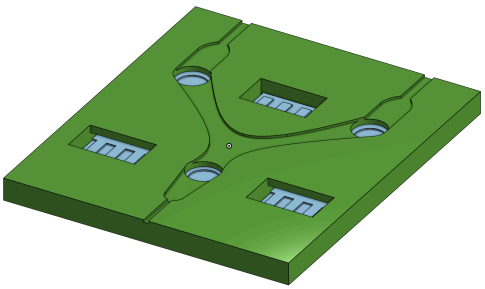
The transduction from current to digital measurements is currently realized through commercial electronics chips :

- A **LMP91000** from Texas instrument, which transduce the current to a voltage value.
- An **ADC121C027**, again from Texas instrument, which converts the voltage value to a digital measurement.

If the results are not precise enough, there is still room for improvements, as a dedicated voltage reference was not given to the ADC and we still can also choose a more pricey transducer and ADC, but that would come at a price.

3. Cartridge technology

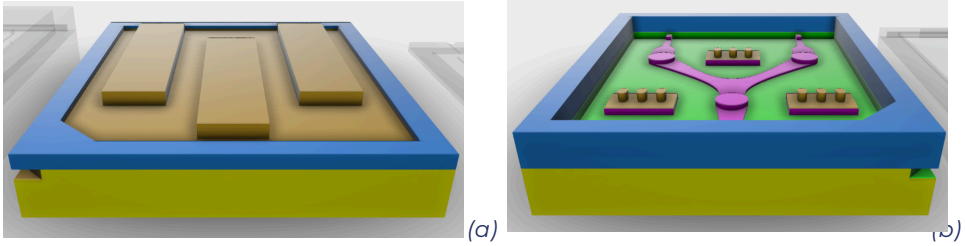
The microfluidic circuit was designed to conduct interstitial fluid through the various sensing stages of the biosensor. The dimensions and shape of the circuit were optimized to minimize the overall volume. The volume of the circuit is 105 µl, equivalent to the volume of the test samples. The corners of the circuit are rounded to minimize fluid retention in the channels.



	dimensions (mm)
sensing area diameter	7
channel height	0.2

diag.1: 3D model of the microfluidic circuit and electrodes in a single block

The microfluidic cartridge is made of PDMS (Poly-Dimethyl-Siloxane), the most commonly used polymer in the field. It is produced by polymerizing PDMS in a photosensitive resin mold printed in 3D. The cartridge consists of two parts: *(a)* The first part is a plate into which the three electrodes are inserted. *(b)* The second part contains the microfluidic circuit and the holes for connecting the electrodes. The two parts are fixed together with silicone adhesive to ensure the block's seal. The final block measures 6x7 cm, matching the surface area of the biosensor.



(a) *(b)*

Resin molds of the two sections of the microfluidic cartridge (a) and (b)

We opted to use commercial gold electrodes on polycarbonate, which limits the interaction possibilities between the circuit and the electrodes. This presents a risk of leaks after multiple uses of the sensor. To improve the circuit's seal, we should use a 6x7 cm glass plate on which the gold circuits of the three electrodes are directly printed. This glass plate would directly replace the first part of the cartridge *(a)*. The second part *(b)*, made of PDMS, would be bonded to the first glass part using a plasma cleaner, ensuring a permanent seal.

4. Reader instrument

We chose to develop our own electronic chips instead of using an already existing one to improve our specificity. That is why our system was built from scratch, allowing us to select each component specifically for our purpose.

We designed the architecture of the PCB delivering a prototype for testing which includes many options.

The electronics of our system have been conceived to be highly deployable. The board has been designed to include many removable options such as bluetooth, on/off switch, leds, programmation connector etc. By taking out those options, the chip's size and cost can be reduced without any additional work.

Only the necessary components have been used on the system which makes it highly competitive in terms of cost. Moreover components that can be bought in large quantities were selected to facilitate a bigger production of our prototype.

Developing our own chip offers several advantages :

- A deep understanding of the entire functionment of the sensing system
- A increased deployability
- No unnecessary options on the chip : only useful features and peripherals

For the measurement electronics we used a Analog Digital Converter with 12 bits encoding and a current sensor with up to 5µA of precision.

Those components could be replaced by more precise sensors such as Analog Device AD5940 which proposes up to 50pA of precision current measurements and integrates 16 bits ADC, but for our application electronic sensors are not limiting measurement precision.

Moreover using this all-in-one chip increases the cost and complexifies the chip making it harder to debug, test and program.

We can also add additional filters on the current sensor without additional work as the selected chip allows extra-filtering and 2 dedicated pins are available on the prototype board.

Originality

1. Team's part

This year, our SensUs team is introducing an innovative enzymatic sensor for creatinine detection, showcasing our biology expertise from AgroParisTech. We collaborated with students from CentraleSupélec and École Normale Supérieure to integrate advanced electronics, microfluidics, and software features.

Initially inspired by an aptameric sensor, we chose to develop an enzymatic sensor that generates an electric current through creatinine degradation. Our device uses four enzymes immobilized on a chitosan gel, applied as a thin layer to a gold microelectrode via electrodeposition. This multi-enzyme approach enhances specificity and sensitivity, with the chitosan gel ensuring stable enzyme immobilization and improved conductivity, while the gold electrode provides efficient signal transduction. A custom microfluidic circuit, developed with École Normale laboratories, guides the liquid for quick detection.

To refine our prototype, we consulted experts and tested the design in AgroParisTech and Micalis Institute laboratories, supervised by Dr. Vincent Sauveplane and supported by UMR Bioger researchers.

We are excited to present this promising creatinine detection prototype at the contest, combining an enzymatic pathway with precise detection and multiple measurements.

2. Supervisor's part

The students of AgroParisTech led the formation of the team. They successfully involved students from Telecom ParisTech and the École Normale Supérieure, creating a multidisciplinary team with skills in biology, microfluidics, and electronics to build their biosensor.

The original idea was to synthesize and use aptamers that specifically bind to creatinine, causing a conformational change in the aptamer and producing an electrical signal proportional to the creatinine concentration in the sample. However, the cost of synthesizing aptamers and the uncertainty of their conformational change upon binding to creatinine led the team to change their strategy. After consulting experts in the field (Mr. Vincent Noël and Ms. Nicole Jaffrezic), the team decided to focus on creating a biosensor based on an ambitious sequence of four enzymatic reactions, leading to the release of electrons that generate a signal captured by an electrode within a microfluidic network. All technical decisions were made by the team with logistical support on protocols from Ms. Jaffrezic. They chose to immobilize the enzymes in a chitosan gel deposited by electrodeposition on the electrode, followed by enzyme immobilization with glutaraldehyde. The electrode is positioned within a microfluidic circuit entirely designed by the students.

Translational potential

1. Introduction

It was with the aim of producing a continuous biosensor that could help in the care of kidney disease that we built our prototype and the company that could commercialize it. As the alarming figures in WHO reports show, chronic kidney disease has recently become a major problem in human health (by 2022, 10% of the world's population will be affected by kidney failure, according to the WHO), and will continue to be so in the future. Indeed, the GBD project estimates that by 2040 chronic kidney disease will be the 5th leading cause of death worldwide. These figures are all the more worrying as we know that chronic kidney disease is still poorly diagnosed. With this in mind, our team set out to produce a continuous biosensor that would best meet the needs of patients and physicians, while at the same time being easily marketable. We were able to contact a number of players who helped us improve our project in a number of ways.

2. Stakeholder desirability

Customers and stakeholders

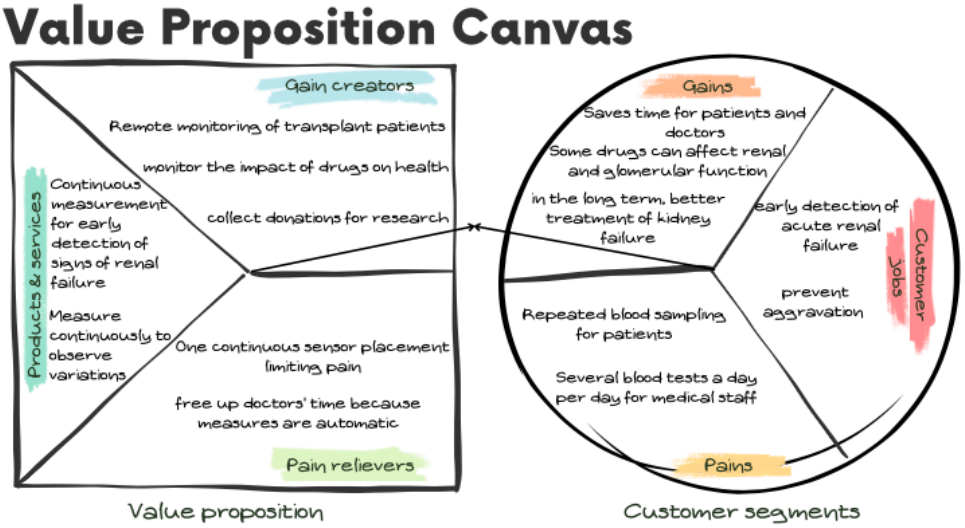
Potential customers for our product are mainly those involved in kidney disease: patients, doctors, nurses, hospital management, insurance companies and researchers. They are looking for a device offering continuous, rapid measurement of clinical signs, in particular creatinine, to detect kidney damage. The product should simplify measurements, saving time for both nursing staff and patients. The aim is to provide each person concerned with clear, precise information on their state of health, accessible via a mobile interface. This will enable patients to monitor their condition and easily transmit data to their doctor, who will be able to react quickly, reducing treatment time and avoiding serious cases. In addition, limiting the number of serious cases benefits insurance companies and hospital management, by reducing costs and the need for beds. The data collected by this product can also contribute to research into chronic kidney disease. However, it is essential that the sharing of such data remains limited to players with no economic interests, in order to preserve ethics and confidentiality. For example, insurance companies may be tempted to increase charges for patients showing clinical signs of kidney failure.

This information should not be passed on to insurance companies or private research centers. Furthermore, it is crucial that this data remains confidential and is not divulged by medical staff. In short, our product positions itself as a central link between the various players in the medical field, while ensuring the protection of patient data.

Value proposition

The market for kidney disease products, estimated to reach 13 million USD by 2023 with rapid growth (CAGR of 7.29%), is dominated by companies such as Thermofisher Scientific and Abbott Laboratories. These major companies mainly offer single-use creatinine assay kits, which do not allow continuous measurements. Our product stands out for its continuous biosensing capability and ease of use via a dedicated application. It can also be used to support research. To date, we have found no patents to limit our development, offering a unique opportunity on the market.

Revenues will be generated by the sale of the biosensor and the associated application, with a business model based on regular replacement of the biosensor, guaranteeing financial stability. We have already developed a biosensor for repeated measurements, and our future objective is to design a patch integrating all functionalities. This patch will be compact, lightweight, easy to change, and will provide accurate, continuous information. The application interface, connected via Bluetooth, will make this data easy to understand for patients and doctors alike, making use of the product practical and effective.



Support

The data required for the market development study were found either in the literature or by interviewing Pr Julien Hogan, a nephrologist working at Robert Debré's hospital. It enabled us to better identify the interests that people working with our product might have. This interview also enabled us to gain a better understanding of patients' expectations, as well as the factors that could cause creatinine concentrations to vary, such as certain medications.

3. Business feasibility

Key resources

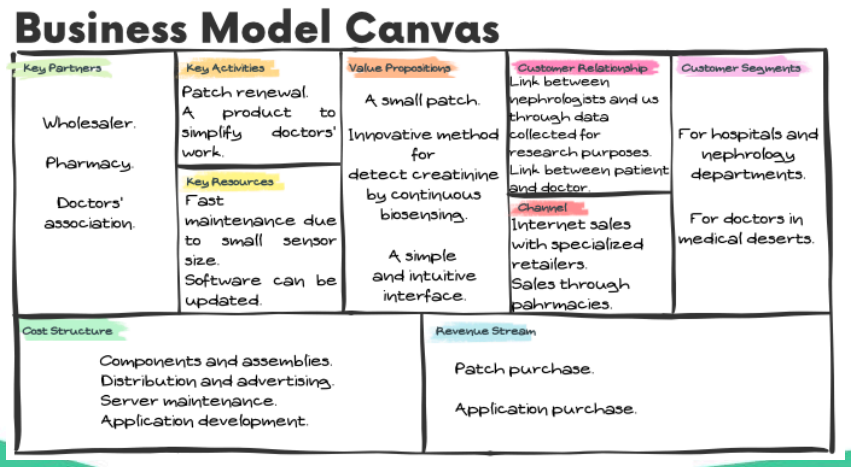
To produce our prototype, we will need a team focusing on the design of the biosensor, so that all the necessary elements are on the part of the human body. A team of developers working on the application to retrieve and present the recovered data will also be needed. In addition, a group will be specialized in the production and distribution of the biosensor, given that we intend to use the major pharmaceutical distributors to distribute our product. We would also like to have a research team that can work on improving the sensor by adding other measurement tools so that we can obtain a multitude of data on a person's physiological conditions from a single sensor.

Key activities

Our marketing strategy is based on the three groups that will promote our sensor: doctors, pharmacists and specialized retailers. First of all, the product will be introduced to doctors as they know which patients our technology will benefit the best. Pharmacies and hospitals are next in line for the marketing campaign as they have the greatest added value. We also want to patent the biosensor technology, and in particular the rights for the phone's app, we are going to code. That way our product will be protected from every side.

Key partners and sustainability

Our key partners will be wholesalers specializing in pharmaceuticals. Our aim is to provide them with a product that they can then distribute on a profit-making basis. In addition, we want to produce a biosensor with a reduced environmental impact. The design is therefore based on the idea of making a product that measures continuously over a long period, so as to replace daily blood sampling, which requires sterile equipment and therefore a lot of plastic. Given that the perishable resource in the biosensors is the gel and enzymes, we could set up a biosensor recovery system that would wash the biosensors and recycle the microfluidic system to make a new biosensor.



4. Financial viability

Costs projection

The current prototype's cost is largely due to electronic components, making it relatively inexpensive when scaled for production with proper investment and sourcing. The cost per sensor is about €120.5, with €20 for electronics, a cost that's hard to reduce, and €102 for printing the microfluidic circuit, which could decrease significantly with large-scale production and a suitable 3D printer. Chemical components, including enzymes and chitosan gel, are minimal at €0.50 per gel.

A notable expense is the manual production of the gel and its electrodeposition, requiring skilled scientists. While automation could reduce this, we've estimated an additional €250 per sensor for this labor, bringing the total to €370.

Each sensor can conduct about 10 measurements before requiring a gel replacement, costing €3, leading to a per-use cost of around €0.30. With a one-year lifespan for electronic and microfluidic components, the depreciation cost is about €1 per use, making the overall per-use cost €1.30—competitive for this device.

To scale commercially, investing in machinery for electronics and microfluidics would drastically lower costs. Further research is needed to optimize the gel and develop user-friendly cartridges, reducing production costs and boosting competitiveness. The current development cost is approximately €6,000, with future improvement costs potentially reduced to €4,000, plus personnel fees. Eventually, our main idea would be to develop a sensor in the format of a patch, for a similar price, but with longer-lasting cartridges on a portable device.

Sales price

To ensure profitability, a pricing strategy of at least 20% above production costs is recommended, covering manufacturing expenses and team salaries. Given the sensor's specialized market, a selling price of €500 would not only ensure profit but also provide funds for further R&D. Replacement gels could be priced at €5, balancing affordability for users with sustainability. These prices would eventually be arranged for a portable device, with longer-lasting cartridges, to be balanced with the production costs.

We also plan to monetize the data generated by the sensors, offering valuable insights to researchers and offsetting software development costs. While still in early stages, this strategy could enhance the product's value and open new revenue streams.

By combining direct sales with potential data and software revenue, we aim to create a sustainable business model that supports ongoing innovation and growth in this field.

Evaluation of the Market

1. Outline of the Total Available Market's Characteristics

The market for creatinine detection is large and expanding; projections for the year 2024 range from 1,042 to 1,250.4 billion USD. The two main test types used to segment the

market are blood and urine testing. The market is further segmented into equipment and consumables, which include test strips, cartridges, and clinical analyzers. For precise measurements, hospitals and labs employ clinical analyzers such as the Roche Cobas series; for at-home and point-of-care testing, test cartridges and strips are available.

2. Future Market Outlook

According to projections, the market for creatinine tests is expected to expand between 2024 and 2032 at a Compound Annual Growth Rate (CAGR) of 13.66% to 17.4%. An aging global population, an increase in the prevalence of renal conditions, technological improvements that improve test accessibility and accuracy, and a rise in health consciousness are all significant growth factors. Demand for home test kits and their integration with telemedicine systems is rising in the industry. Because of its large healthcare spending, North America is likely to lead the region, while Asia-Pacific is expected to develop quickly.

3. Overview of the Golden Standard in this Market

Mass spectrometry (MS) is considered the gold standard for creatinine detection due to its high sensitivity, specificity, and accuracy. Although the speed

and cost-effectiveness of enzymatic and Jaffe techniques make them popular in clinical practice, they lack the specificity of MS and are more prone to interference. Sample preparation, centrifugation, and analysis utilizing LC-MS/MS equipment are standard steps in the MS process. MS has struggled to be adopted despite its greater performance because of the expensive cost of the instrument, the demand for expert operators, and the need for frequent maintenance. Nonetheless, its accuracy and reliability in quantifying creatinine make it invaluable for both clinical diagnostics and research applications, ensuring its position as the gold standard in creatinine identification and quantification.

Revenue streams and business strategy

At this point, the revenues from the business of the biosensor are expected to be made out of the selling of sensors and the data produced by our devices. For the selling of the sensor, the pricing as proposed in part 1.4.2 ensures a margin around 10% per device, with the other 10% differentiating the price of production to the selling price being used as back-up for covering additional costs for distribution and commercialisation.These 10% represent thus a way for the company to develop if the sellings are good, with additional income from selling data, which is still to be estimated. The calculation of the break-even point, from the usual formula [8] is then between 0.9 and 1.

Team and support

Our project would not have become a reality without the dedication and support of many individuals and organizations.

Team Contributions:

We are proud to highlight the members of our team who played crucial roles in the development of our biosensor:

Electronics Team: Yanis Gomes, Matthieu Pringent, Basile Plus

Experimental Team: Dianyi Jiao, Axel Garbe, Axelle Lorimier, Lucie-Garance Barot, Raphaël Teissier, Lisa Schmitt, Kiran Souprayenmestry Rangapamodely, Lucie Guerin, Guillaume Centene-Guglielmi, Brieuc Dervyn, Eva Legrand, Walid Ben Rahal

Entrepreneurial Team: Brieuc Dervyn, Lucie-Garance Barot, Axel Garbe, Raphaël Teissier

Special Thanks:

We extend our heartfelt gratitude to our outstanding team coach, Vincent Sauveplane, whose guidance and patience were really precious for making our biosensor reality.

We also wish to thank the researchers who generously supported us throughout this journey: the team led by Bruno Le Piouffle in microfluidics of the ENS Paris-Saclay, the research team that graciously hosted us in their laboratories during the summer : Jean Frédéric Audibert , and Nicole Jaffrezic for her invaluable assistance.

We would also like to acknowledge the contribution of our three schools—CentraleSupélec, ENS Paris-Saclay, and AgroParisTech—, and more widely to the university Paris-Saclay. Their close proximity on the Saclay plateau and training programs enabled us to assemble this interdisciplinary team.

Financial Support:

Our sincere thanks go to the institutions that funded our efforts and made our project possible: La Compagnie Financière de Haute-Joux, Université Paris-Saclay, Fondation AgroParisTech, and the UMR Microbiology and Molecular Genetics of Micalis.

Finally, we express our deep appreciation to the organizers of the contest, who provided us with the opportunity to work on a project that integrated both entrepreneurial and scientific dimensions.

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