Team Results Document

PULSe

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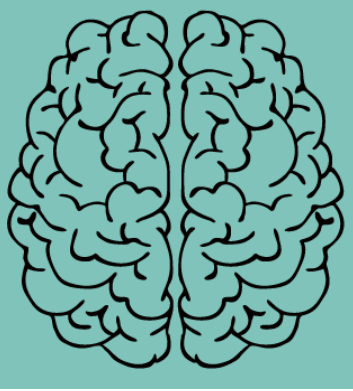
KU LEUVEN

SensUs



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1. Abstract

The Point-of-Care University of Leuven SensUs team (PULSe) is representing KU Leuven at the SensUs 2023 competition. This year, we are proud to present EISY, a self-powered point-of-care (POC) and user-friendly biosensor for rapid detection of glial fibrillary acidic protein (GFAP) in plasma during a traumatic brain injury (TBI).

Our biosensor allows for ultrasensitive electrochemical detection of GFAP using screen-printed electrodes (SPE). The SPE is embedded into a disposable microfluidic cartridge, making use of our in-house-developed self-powered microfluidic pumps, which allow for fully automated and autonomous sample processing. The SPE is functionalised with GFAP antibody (Ab) coated gold nanoparticles (AuNP). Consecutively, electrochemical impedance spectroscopy (EIS) is used to determine the GFAP concentrations, as the resulting impedance from an electroactive species is correlated to its concentration. All these elements are brought together in a one user friendly tabletop device, which comes with a phone app to allow for patient follow up by specialists.

With this device, we hope to fulfil the current needs of both hospitals and first-line care takers regarding traumatic brain injuries in their daily work. Furthermore, we introduce a concrete business model to explore the financial future of the biosensor.

2. Biosensor system and assay

Over the course of the following paragraphs, an overview of the proposed biosensor for GFAP is discussed. An illustrative overview figure is provided in Figure 1.

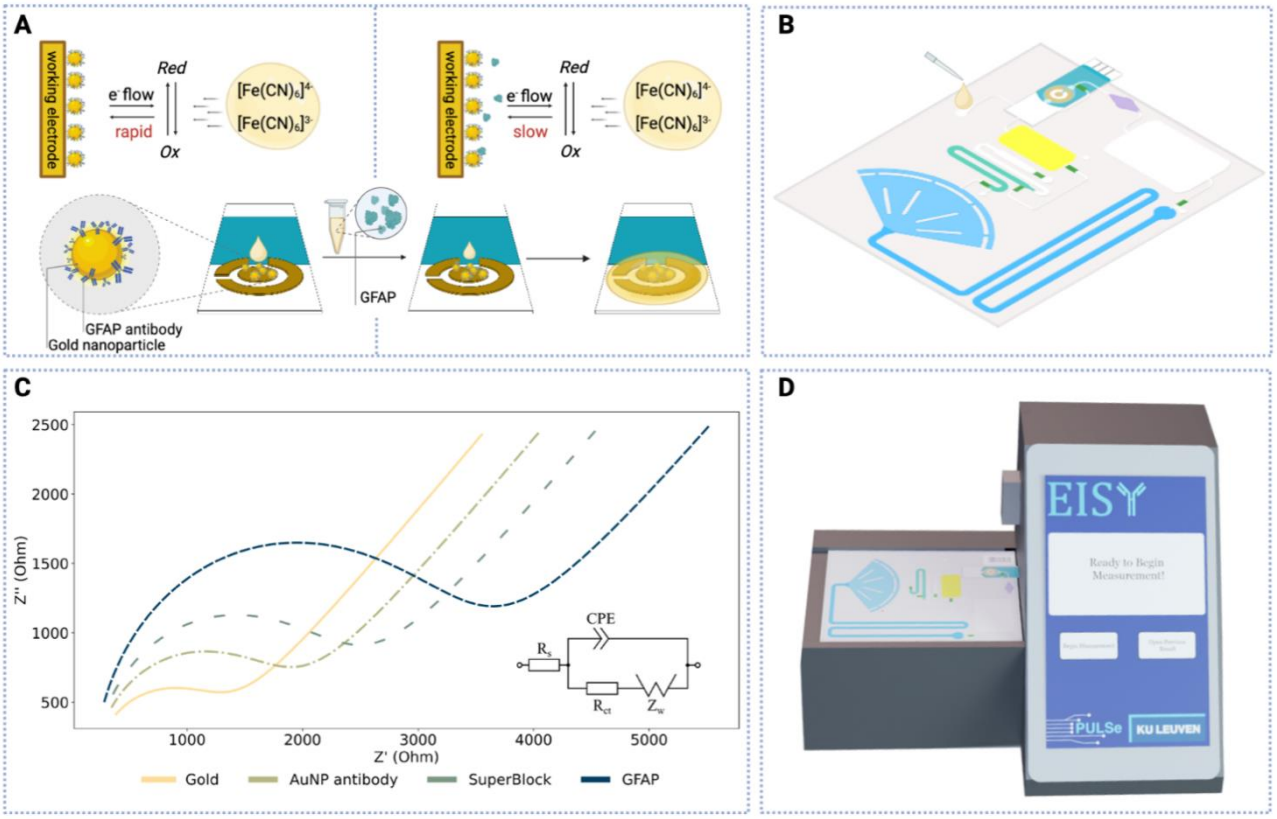


Figure 1: Schematic diagram of the label-free electrochemical biosensor based on antibody-antigen (Ab-Ag) complexation. *A) Diagram of molecular recognition assay, which influences flow of electrons (e^-) originating from a reductant (Red) – oxidant (Ox) couple (ferro/ferricyanide), B) Graphical representation of the autonomous (i)SIMPLE cartridge. C) Read out of the impedance over a frequency range (1.1 – 200,000 Hz) and fitted against an equivalent Randles circuit using the PICO EmStat. D) Rendering of the final device*.

2.1 Molecular recognition and assay reagents

Specific binding of GFAP to the sensing surface in a complex matrix like plasma is done using **functionalised AuNP with GFAP capture antibodies** (83cc, Hytest, Finland) through electrostatic adsorption (Ruiz *et al.,* 2019). According to literature, adsorption of antibodies on AuNP can give a higher rate of bound antibodies in comparison to a covalent technique *(*Oliveira *et al.,* 2019). The use of AuNP allows for a higher surface effective area, improved selectivity and catalytic capabilities *(*Zhang *et al.,* 2020). Secondly, the immobilisation process on the SPE is carried out by incubation of the working electrode (WE) with the functionalised AuNP (Figure 1A). Lastly, a blocking step is performed using SuperBlock™ (Thermo Fisher, USA) to prevent nonspecific binding to the non-functionalised parts of the surface. For all of these steps, a homemade incubation cell was made from PMMA (Appendix 9.1). This cell allows for only the WE to be exposed for functionalisation and prevents reagent evaporation, which is often seen as a drawback of drop casting as it favours the formation of ring like patterns (Kaliyaraj Selva Kumar *et al*., 2020).

2.2 Cartridge technology

To improve user-friendliness, the functionalised electrode was incorporated into a disposable, robust and low-cost microfluidic cartridge based on the **infusion Self-powered Imbibing Microfluidic Pump by Liquid Encapsulation (iSIMPLE)** technology (Dal Dosso *et al*., 2018). The main characteristic of this approach is the on-chip pumping mechanism, enabled by capillary imbibing through a porous material (Whatman filter paper grade 598). After activation, this principle results in the autonomous actuation of the working liquid. By creating a positive or negative pressure in the channels, liquids can be pushed or pulled through the chip respectively. Our developed cartridge

allows for **fully automated and autonomous performance of all steps** (Figure 2). The running buffer solution consisting of phosphate buffered saline (PBS) also incorporates a redox probe, enabling the solution to (1) first serve as a washing step (i.e. to rinse the sample and prevent nonspecific binding), and (2) provide a means for electrochemical readout later on.

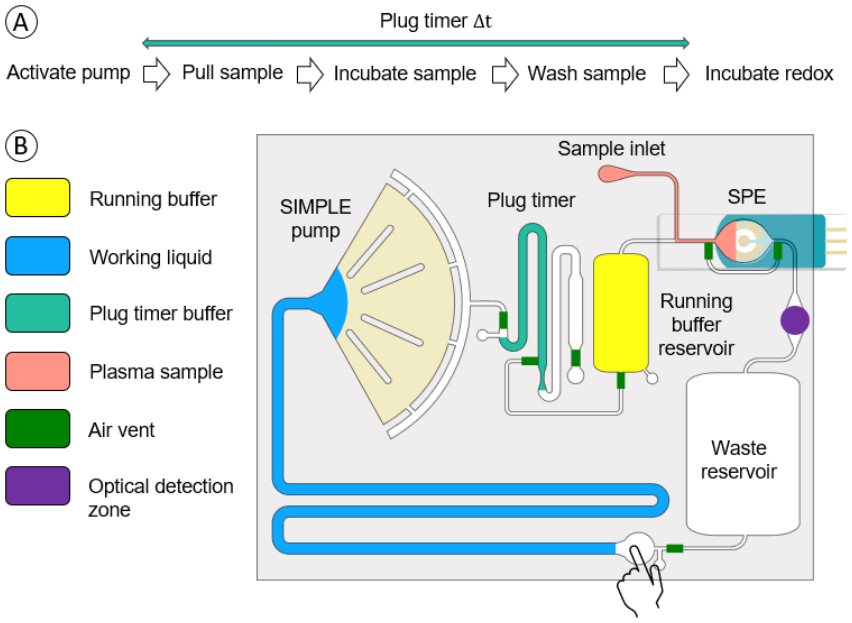


Figure 2: Graphical representation of (A) the workflow and (B) the parts of the SIMPLE cartridge.

Additionally, a dye (food colouring) was added to the running buffer solution, enabling **detection when the probe is incubated on the electrode facilitated by an infrared (IR) LED and IR phototransistor pair**. The specific voltage difference measured, as the coloured buffer solution flows in and out of the chamber between the LED and the phototransistor, indicates that the redox probe is incubated on the electrode. This triggers the initiation of the measurement process (Appendix 9.2).

2.3 Physical transduction

The use of a gold WE in combination with the reader and accompanying hardware (PalmSens, The Netherlands) allows for the utilisation of a myriad of different electrochemical detection techniques. Among these, EIS is favoured due to its **label-free**, **sensitive**, and **fast detection** capabilities. EIS involves applying a small alternating voltage across the electrode over a specific frequency range, during which the impedance is determined and plotted on a Nyquist plot.

Subsequently, the acquired data is fitted against an equivalent circuit. An often-used circuit for biosensors is the Randles cell (Figure 1C). This cell includes several components: a solution resistance (R_S), a double-layer capacitor (C_{DL}), a charge transfer resistor (R_{CT}), and a Warburg element (Z_W). The charge transfer resistor (R_{CT}) shows concentration-dependent behaviour, as it represents the flux rate of the electrons coming from the redox probe, ferro/ferricyanide in this sensor, to the electrode surface. This flux rate is heavily affected by the formation of Ab-Ag immunocomplex, which acts as a physical barrier causing a lowering of the flux (Bigdeli *et al.,* 2021; Moya, 2013). The difference in estimated R_{CT} before and after antigen binding is used to establish the calibration curve. **To enable rapid on-field measurements**, each chip is calibrated in the laboratory to obtain blank R_{CT} values. These values are then communicated directly to the device via a QR code scanner.

2.4 Reader instrument and user interaction

The user will mostly interact with a graphical user interface (GUI) displayed on a touch screen. The GUI has been chosen to be as minimalistic as possible by reducing the number of buttons, resulting in an **easy and intuitive interface**. The steps represented in Figure 3 should be followed for each measurement.

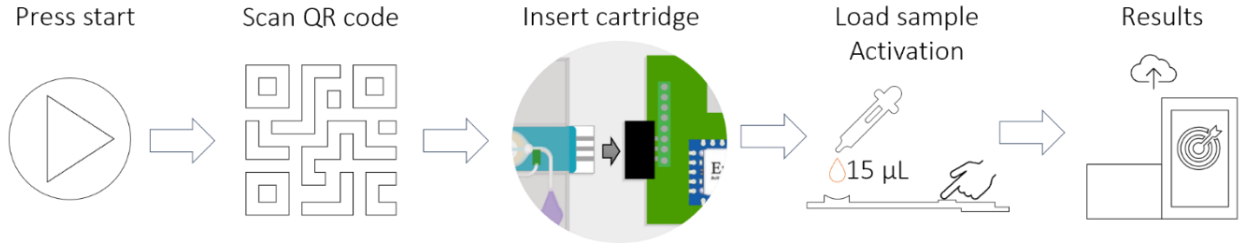


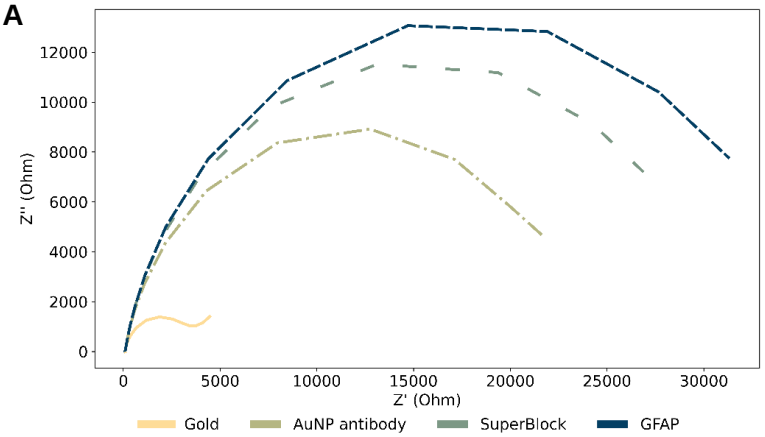
Figure 3: Graphical representation of the workflow from start until results.

3. Technological feasibility

3.1 Molecular recognition and physical transduction

GFAP detection was accomplished using EIS. After each of the functionalising steps, an increase in fitted R_{CT} values are expected as the physical barrier present on the WE increases. The latter can be observed in Figure 4 (A and B) as the diameter of the fitted semicircle in the Nyquist plot is directly related to the R_{CT} value of the corresponding Randles circuit (Bigdeli *et al.,* 2021). Thus, confirming both the deposition of nanoparticles onto the WE and a correct blocking step using SuperBlock™. Additionally, **GFAP spiked buffer samples show a proportional increase** (Figure 4 (C and D)), while it seems that buffer and plasma alone did not cause any increase (Appendix 9.3). The latter suggests **no nonspecific binding of the buffer** with our electrode surface.

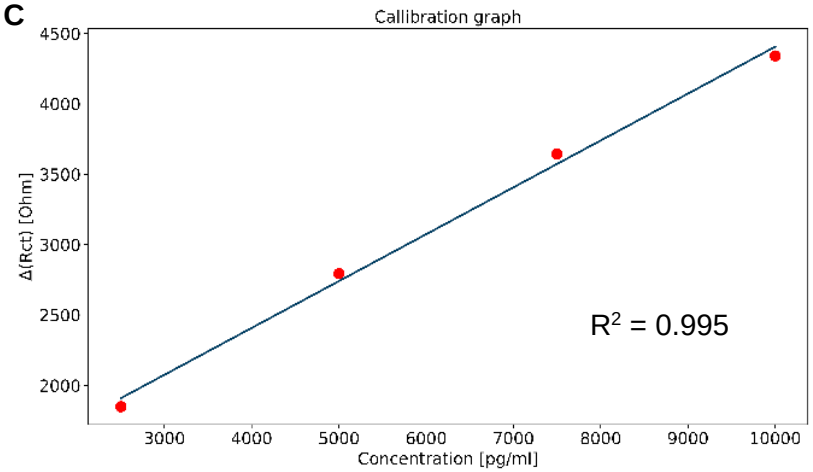
A



B

Molecular layer	R_{CT} (Ohm)
Bare	2702.91
AuNP Ab	8148.34
Superblock	9905.81
GFAP	13069.13

C



D

[GFAP]	ΔR_{CT} (Ohm)
2500 pg/mL	1850
5000 pg/mL	2794
7500 pg/mL	3163
10000 pg/mL	4846

***Figure 4: Feasibility of the assay**. **A)** Nyquist diagram showing the EIS spectra of the WE over a course of different functionalisation and detection of 10000 pg/mL GFAP concentration in water. **B)** Table of the corresponding fitted R_{CT} values for the different functionalisation layers. **C)** Dose response calibration curve of GFAP **D)** Table showing the ΔR_{CT} values corresponding to four distinct GFAP concentrations.*

We are currently optimising the assay to allow better deposition of the Ab-AuNP onto the WE, allowing us to reach a higher sensitivity. Lastly, we are also in the process of producing a Limit of Detection (LoD).

3.2 Fluidic cartridge

To allow the user to perform the above measurements in a user-friendly, rapid, robust, equipment-free and affordable manner (all key characteristics of a successful POC device), the functionalised electrodes were integrated into a fully automated microfluidic cartridge (Ahmed et al., 2016; Konwar & Borse, 2020). This novel module of the (i)SIMPLE toolbox, was first tested against the state-of-the-art protocol, verifying the impact of the cartridge on the electrochemical measurements. Hereby, we compared respectively depositing the redox probe onto the electrodes, by flowing it into a **microfluidic channel or by depositing it** using a micropipette. Where the former resulted in an increase of R_{CT} and R_S values compared to a bare SPE. Next, to enable detection of running buffer by the LED and IR phototransistor pair a coloured dye was added to the buffer solution. This resulted in an increase in R_{CT} value, yet no increase in R_S was observed. Nevertheless, the implementation of the cartridge nor the addition of a coloured dye change the essential **characteristics of the EIS readout on a gold SPE and thus the former stated**

equivalent circuit remains unchanged (Appendix 9.4). To further assess the influence of the cartridge on the electrochemical measurements (e.g. if it alters the measurements during functionalisation and the respective sensitivity), additional experiments will be performed.

To **enable sample and redox buffer incubation** on top of the SPE, an air bridge composed of two hydrophobic barriers was employed, effectively partitioning a volume of 8 µL in the chamber above the electrode (Figure 5A, Appendix 9.5). To **tune the duration of sample incubation**, a plug timer, inspired by the design of (Vloemans et al., 2022), was implemented (Figure 5B). For the targeted incubation time of 5 minutes and a pump speed of 15 µL/min, the timer channel consists of two equal segments (each 75 µL) with respect to an air connection. Initially, the first segment of the timer is loaded with buffer. Upon cartridge activation, this buffer starts to flow into the second segment. Once all liquid was transported from the first to the second segment, an air connection between the (i)SIMPLE pump and the running buffer reservoir is established (Figure 2), allowing the buffer solution to enter the SPE incubation chamber. The buffer solution will be delayed by five minutes compared to the sample pull, ensuring the required incubation period. Upon entering the SPE incubation chamber, a volume of 8 µL running buffer will be parted.

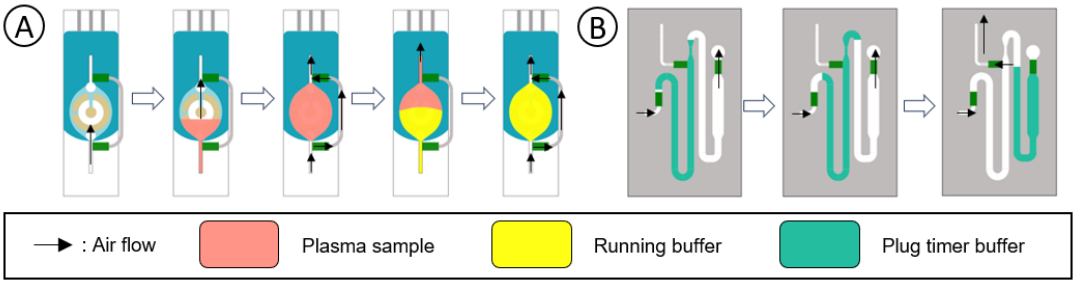


Figure 5: Working principle of A) *the air-bridge-mediated SPE incubation chamber, and* ***B)*** *the plug timer.*

While not employed in the present chip design, the inclusion of microneedles (Van Hileghem et al., 2022) and a plasmapheresis unit remains a potential enhancement. This prospective integration positions the chip to evolve into a comprehensive, user-friendly, and autonomous sampling device for electrochemical readouts.

3.3 Reader instrument

In order to integrate the cartridge with the device, a small slot has been prepared on the side, allowing for the cartridge to slide into (Figure 6A) (Appendix 9.7). A **QR-code scanner** is mounted right above the cartridge allowing to retrieve individual blank (before sample incubation) R_{CT} value so the difference in R_{CT} can be measured, which is necessary to determine the concentration via the calibration curve (Appendix 9.6). Lastly, the results can be sent to a cloud database via a **smartphone application** (Figure 6B). This holds the potential to promptly store results in a hospital database, leading to procedure acceleration.

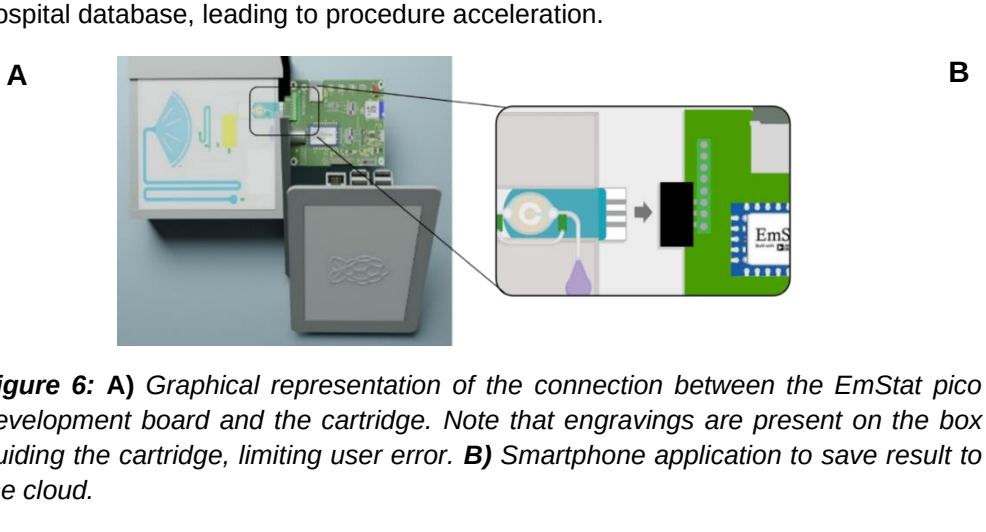


Figure 6: **A)** *Graphical representation of the connection between the EmStat pico development board and the cartridge. Note that engravings are present on the box guiding the cartridge, limiting user error.* ***B)*** *Smartphone application to save result to the cloud.*

4. Originality

4.1 Written by Team Captains:

After an extensive literature review, the most promising biosensors technologies for TBI detection available are currently: electrochemical amperometry detection (Abbot), optical detection (fluorescence) based on paramagnetic particles (Simoa Bead Technology), and chemiluminescence (Banyan BTI ™). With regards to the electrochemical label-free biosensor for GFAP detection, earlier literature report AuNP and L-cysteine for Ab functionalisation, as well as EIS for GFAP detection (Ozcelikay *et al*., 2022*)*. However, to the best of our knowledge, there has been no report of a label-free assay using electrochemical detection incorporated into a fully autonomic microfluidic platform.

For this competition, we developed a label-free detection assay directly on a SPE incorporated into a self-powered microfluidic chip. Integration of a SPE into a microfluidic chip is a completely original and novel idea as it enables a highly complex assay to be conducted automatically on the electrode, generating results in real-time. The microfluidic chip is powered by a single-push-activated paper pump ((i)SIMPLE), with working liquids and buffers already inside the chambers and channel. The microfluidic chip based detection minimises and simplifies laboratory steps, hence shortening the time for results. To top it off, we have assembled a portable readout system that emphasises a user-friendly interface, with ease-of-handling and a ready to use instrument.

As a team, we started our work by reading the important aspects of GFAP detection, the currently available techniques, and the challenges encountered when working with complex matrices such as plasma. With guidance from our coaches, we narrowed our ideas over the span of a few months to the most viable and original ones. From there on, we worked, planned, and designed our experiments mostly independently, but with the guidance of our coaches during weekly meetings. Our team then worked on conceiving the (i)SIMPLE adapted for the bioassay design, optimising the functionalisation of SPE surface, and its integration into our own reader instrument and the potentiostat. Unfortunately, our ambitions and the challenges we encountered limited the performance of the final product within the time frame of this competition. However, the present concept is backed up with literature and preliminary data of various experiments that we have performed during this year.

4.2 Written by Supervisors:

The work presented here by our PULSe team, shows a first proof-of-concept of what has the potential to become a beyond-the-state-of-the-art biosensor, as the team succeeded for the first time in the successful integration of electrochemistry with our in-house-developed (i)SIMPLE technology. To achieve this ambitious goal, the students developed a novel innovative SIMPLE module: an SPE incubator chamber. Herein, the team successfully obtained leak tight integration of the SPE into a (i)SIMPLE cartridge, which assures complete electrode coverage when sample is flown through the channel, a necessity for accurate electrochemical measurements. Additionally, this was extended with an air-bridge and a plug timer, concepts previously established by Vloemans *et al*., allowing to achieve precisely timed incubation of the sample on top of the SPE. Although the incorporation of an electrochemical readout into a microfluidic platform has been reported in literature before (Nix *et al*., 2022; Salahandish *et al.*, 2022). the unique combination with our (i)SIMPLE technology makes this biosensor the first fully self-powered biosensor of its kind. Additionally, our sensor boasts a user-friendly readout accompanied by a self-calibration feature accessible through QR code scanning. The device is outfitted with Bluetooth connectivity, enabling the potential transmission of real-time results to either a physician or a hospital database. To delete user-dependency, a cartridge-embedded optical emitter/receiver ensures timely and precise measurements.

On top of this, the developed biosensor comes with a user-friendly established assay implemented on a novel device. In the process of developing the presented biosensor, the students were guided by a motivated group of 4 PhD students and myself (the supervising professor). Residing in this support system, providing them with valuable knowledge and guidance throughout the whole process, the students were encouraged to work independently and come up with innovative concepts themselves. From the start, the students have presented themselves as a strong team, creative, hardworking, and eager to learn, which eventually lead them to design the innovative biosensor presented here today. Although significant further optimisation will be required, the preliminary results show great promise for the future.

5. Translation potential

5.1 Business model

<table>
<tr>
<td rowspan="2">Problem
Subjectivity and limited specificity of Glasgow Coma Scale → unnecessary CT scans

CT scans
• Expensive
• Radiation exposure
• Insensitive
• Long time until diagnosis

Guidelines for patient monitoring a not clear or missing</td>
<td rowspan="2">Solution
Accurate diagnosis of all types of TBI using GFAP

Screening procedure to determine the need for CT scan
• Cost-effective cartridges
• Reduces radiation
• Accurate
• Fast results

Long-term monitoring using anti-GFAP autoantibody</td>
<td>Unique Value Proposition
• Fast and accurate results (within 15min)
• Cost-effective (€40/cartridge)
• No professional training or lab equipment needed
• No sample preparation
• Patient monitoring

High-level Concept: Accurate indication of the severity of a head injury. Our sensor enables medical professionals to take the right course of action with minimal training and allows physicians to easily follow-up patients after discharge.</td>
<td>Unfair advantage
• Spin-off KU Leuven
• Unique cartridge technology from the university
• Access to technology, funding, guidance, and legal advice</td>
<td>Customer segment
• Professional healthcare services: hospitals in Belgium, and EU
• General point-of-care: First aid personnel, retirement home, sport institutions, military

Early adopters
Local hospitals</td>
</tr>
<tr>
<td>Existing alternatives
• Neurological exam
• Imaging tests (MRI, CT)
• Other test: Blood test: The Banyan Brain Trauma Indicator™ (BTI), S100B test, i-STAT TBI plasma cartridge (Abbott), Quanterix Simoa assay</td>
<td>Key metrics
• Yearly Sales
• Accuracy</td>
<td>Channels
• Advertising
• Pharma/hospital representative
• Word of mouth
• Medical conferences</td>
</tr>
<tr>
<td colspan="3">Cost structure
Fixed costs: Manufacturing equipment, facility costs, salaries, regulations & patent, R&D cost

Variable costs: Distribution costs, marketing costs, cartridge technology, device costs, clinical trial</td>
<td colspan="3">Revenue Streams
Sensor: bought once (€3000)
Cartridges: each cartridge can be used once, customers can buy new cartridges in packs of five (€40 x 5)
Access to curated user data (€15/ data point)</td>
</tr>
</table>

Figure 7: Representation of the Business canvas model.

5.2 Market description

Traumatic brain injury represents a huge economic burden on the European healthcare sector. In 2020, the global accompanying costs within the healthcare sector due to **TBI were estimated at €400 billion** *(*Van Dijck *et al.,* 2020*)*. The largest contributor to these costs is mild TBI (mTBI) cases, which represent 70-90% of TBI patients *(*Maas *et al.,* 2017). Currently, the gold standard for identifying mTBI patients in need of further intervention is neuroimaging, generally computed tomography (CT) scanning (Maas *et al*., 2017). However, the **use of CT scans is disadvantageous** because: (i) they are expensive, with cost estimates ranging from €190 to €2000 (Paul *et al*., 2015), (ii) they are relatively insensitive, with only 5% of confirmed mTBI patients displaying CT abnormalities (Maas *et al*., 2017). Therefore, many hospitals have opted to use screening methods, like the Canadian CT Head rule, to decide if a suspected TBI patient is in need of a CT scan. Nonetheless, many of these screening techniques are hampered by low specificity, leading to unnecessary CT scans (Bouida *et al*., 2013). This leaves an opening in the TBI-related medical device market for an assay that can: (i) specifically determine if a suspected mTBI patient requires a CT scan and, (ii) can provide a quantitative measure of TBI severity to supplement CT scans in TBI prognosis.

Our biosensor, the PULSe EISY system is able to meet these needs in the market. It will not only help hospitals save costs and resources on unnecessary CT scans but also help TBI patients by providing an additional metric for TBI severity. Figure 7 provides an overview of our business plan in the form of a lean canvas.

5.3 Stakeholder Desirability

The PULSe EISY system is a user-friendly system for easily detecting GFAP concentrations in blood samples. With the use of a blood sample and a specially designed cartridge, the **EISY system works as a POC device that can sensitively and specifically detect GFAP**. This design gives several advantages to hospitals, our primary target customers. The first advantage is saving costs by **avoiding unnecessary CT scans**; previous models have estimated that biomarker screenings begin to save hospitals money when priced at €280, meaning our customers will save at least €240 euros per test with our €40 cartridges (Su *et al*., 2019).The second advantage is that medical equipment, like CT scanners, and staff can be freed up for higher risk patients. The final advantage is a quantitative measure of TBI severity from GFAP plasma levels, which can aid physicians in their prognosis. In addition, our EISY system also offers smaller and rural hospitals the ability to diagnose the severity of TBIs without the purchase of large and expensive scanning equipment.

Because of the myriad of advantages from a TBI biomarker assay, there are several competitors in the market: (i) the Banyan BTI™, a GFAP and UCH-1-based biomarker assay, (ii) the Quanterix Simoa® assay, a GFAP, tau, UCH-1, and NF-L based bioassay, (iii) the plasma-based S100B assay, which is a plasma based S100B assay (iv) the **Abbott i-STAT TBI Alinity assay**, which is an assay detecting both GFAP and UCH-L1 levels in human plasma (Krausz *et al*., 2021). However, the former three of these devices have several major disadvantages that give our device a competitive edge. Both the Banyan BTI™ assay and the Quanterix Simoa® assay suffer from analysis times exceeding two hours (Krausz *et al*., 2021) which makes both devices **difficult to realistically integrate into clinical pipelines** as a screening tool. Furthermore, the S100B assay suffers from low specificity, which is estimated at about 33% (San Miguel *et al*., 2016). This means that the S100B assay results in far too many false positives to be an effective screening tool. Additionally, a Belgian study determined that the use of the S100B assay as a CT screening tool resulted in a loss of revenue to the hospital (San Miguel *et al*., 2016).

This leaves the i-STAT Alinity assay, which is able to accurately detect GFAP and UCH-1 in blood samples in 15 minutes. The i-STAT Alinity assay also has the benefit of clearance from several regulatory bodies, e.g FDA clearance in the US and CE (Conformité Européene) marked in the EU (Abbott, 2023; Abbott UK, 2023). This means Abbott's market share of the TBI device market will be the most difficult to take on. However, the fact that the i-STAT's clearance in both the US and EU is relatively recent, means there is a limited brand recognition of the device. Additionally, the PULSe EISY system has two major advantages over the i-STAT Alinity system: (i) **Our device is point of care**, which allows for a larger range of users (ii) **a lower price tag** of only €3000, compared to the i-STAT's €7000 (Abbott Laboratories, 2011), which will lower the barrier-to-entry for TBI biomarker testing. We will also leverage our status as a smaller and more focused company than Abbott to develop closer and more loyal relationships with our customers. **We will be able to develop close working relationships** between our engineering team and our customers, facilitating a level of customer care that is difficult for a multinational corporation to replicate. Additionally, we will also move towards the development of other biomarker tests. The advantage of our cartridge-based design means that we will be able to extend the prognostic value of our device by designing further cartridges geared towards other acute biomarkers for diagnosis, such as UCH-1, and chronic biomarkers for follow-up, such as anti-GFAP autoantibody (AutoAb[GFAP]) (Maas *et al*., 2017). The inclusion of chronic biomarkers such as AutoAb[GFAP] will allow the device to be not only used in follow-up clinical evaluations, but will improve the quality of our tie in the mobile application. Patients who opt in properly will be able to visualise more detailed information about the time course of their recovery and contribute to further research on what techniques best facilitate rehabilitation from TBI.

We also have several partners and suppliers to help ensure a competitive advantage, including: (i) PalmSens who provides us with our electrochemical measurement system, the PICO EmStat, (ii) Metrohm, who provide us the C223AT SPE electrodes, and (iii) Hytest, from whom we received monoclonal antibodies (GFAP83cc) and recombinant GFAP from.

5.4 Business Feasibility

To support the initial stages of our development as a company, we will use several connections at KU Leuven to secure financial support before transitioning to broader funding sources. We will first secure financial support from KU Leuven investment fund Gemma Frisius and government grants such as the Eurostars funding programme. We will also gather business advice and financial support through the Research and Development Department of KU Leuven. Their guidance and assistance will help us in setting up our spin-off company by offering us entrepreneurial coaching, methods for securing funding, and essential advice on legal and intellectual property matters. We are already in contact with **Francesco Dal Dosso, the Innovation Manager of the MeBioS Biosensors group**, who provided us with guidance and support in developing a sustainable business model. To complete the development of our technology, a team of specialised sub-teams focusing on bioassay, microfluidic design, and readout, each led by PhD students, will focus on developing and optimising the biosensor.

Once we pass our initial stages as a company, we will need to market our EISY system through innovative methods in order to compete with the i-STAT Alinity System. Although our connections to local hospitals, like the university hospital (UZ Leuven, Universitair Ziekenhuis Leuven) will allow us to develop closer working relationships with potential customers. Abbott's status as a multinational company means that it will be difficult to match their hospital

connections. As such, we will also market our product with the use of a tie in application to our device. TBI-diagnosed patients can use the complementary app to track their recovery and to get quick answers to questions about e.g., their daily health. The data gathered from the application can also be used by research institutions to improve their treatments if the patient agrees to be involved in this process. This application will allow us to develop closer ties with the populations affected by TBI, institutions involved in TBI research, and hospitals. It will additionally offer an innovative way for TBI patients to feel more engaged and in control of their own recovery process.

To establish ourselves as a sustainable company, we have taken measures to minimise waste and power consumption of our devices. Our current chip technology has a size of (10 x 8 cm). In future iterations, the size will be reduced to at least (5 x 5 cm). This is of particular importance as these cartridges will need to be disposed as medical waste. Although these chips are made of plastics, **the POC approach to CT screening will mean an overall greater reduction in carbon emissions** from a reduction in use of hospital equipment in unnecessary scans and visits. Furthermore, due to the smaller size of the Pulse EISY in comparison to other diagnostic tools like CT machines, power consumption will be reduced. On average, the operation of a single CT scanner over a year consumes around 26226 kWh, which is near the yearly energy consumption of five households of four people (Heye *et al*., 2020). In contrast, our device's yearly power usage would be 73 kWh, running for 8-eight hours a day non-stop.

In addition to ecological sustainability, we believe that our initiative in making more data available regarding the treatment and diagnoses of TBI will accelerate the development of further cost-effective POC sensors and improve the treatment of TBI patients. By entering the market at the relatively low price point, we will be able to make screening and treatment more accessible to a larger range of people suffering from TBI. This will especially be useful for low and middle-income countries (LMICs). Accessing emergency care in LMICs, particularly in rural regions, remains a major problem due to limited availability and long distances to healthcare facilities. Less than 1% of the population in LMICs have access to ambulances, highlighting the need to focus on prehospital care to save lives and improve healthcare (Kironji *et al*., 2018). **Our device will ease the reliance on ambulances** by providing a user-friendly, and cost-effective biosensor onsite, easing the gap in health inequality between affluent and poor individuals. There are many countries where access to healthcare is severely limited by cost. By using our device, unnecessary costs for CT scans will be reduced (from approximately €2200 to €40).

Figure 8: Diagram of the business timeline. Summary of the 4 phases of our business development: 1) incubation phase, 2) selling in the Benelux market, 3) expanding sales, and 4) expanding portfolio.

In order to successfully achieve this vision, we have broken down our growth as a company into four phases which are summarized in Figure 8.

Phase I: Testing phase and initial sales: During the first three years, we will focus on: (i) optimising the biosensor, (ii) patenting and satisfying regulatory requirements, and (iii) securing connections with local hospitals, allowing us to conduct research with patients to validate our device's clinical utility. To optimise the biosensor, we will devote resources to increasing the specificity and sensitivity of the bioassay as well as developing the readout software.

To patent our product, our legal advisor will first ensure our device has freedom to operate. Once we have collected sufficient data on the efficacy of our device, we will certify our device at the nearest notified body qualified for diagnostic medical devices, DEKRA Certification B.V., before further obtaining a CE mark for our biosensor in accordance with the EU Regulation on Medical Devices (MDR). To satisfy regulatory requirements regarding our data, it will also be ensured that our mobile application data collection is in accordance with the GDPR. We plan to achieve compliance by having users freely consent to data collection and market before their use of the application. During our certification process, our legal and financial advisor will begin filling in international patent paperwork for our specific chip technology for the detection of GFAP, for the diagnosis of TBI.

To secure connections with local hospitals, we will start by exploring **KU Leuven's connections with both the university hospital (UZ) and the KU Leuven TBI centre (Antwerpen)**. In this phase, we will offer prototype devices to the doctors and to their patients a free phone application linked to our device. The application will keep track of user information like their rehabilitation and general well-being. Using the repository built by the mobile application relationships can be built with local doctors and groups affected by TBI.

Our team will consist of biomedical engineers and computer scientists, with consultation by an outside financial and legal advisor. We will continue to operate in KU Leuven's Biosensors group, to save on lab space costs. To adequately fund our staff and research, we will begin with grants from KU Leuven, VLAIO, FWO or EU projects to keep us afloat during this period.

Phase II: Selling in the Benelux market: Once we have achieved a CE mark for our device, we will commercially launch our device and ensure a solid base of local sales in the Benelux region (i.e., Belgium, Netherlands, Luxembourg), allowing us to establish an initially small and focused market to keep better control on sales numbers. At this phase, the marketing and administration officers will entail: (i) travelling in the Benelux region to hospitals and medical conferences to advertise our device, which will primarily be marketed as a TBI screening tool, but with the additional advantage of enabling the monitoring of chronic biomarkers, and (ii) developing marketing campaigns to increase app usage amongst TBI-sufferers. Since we will need dedicated hosting for our app, we will outsource the server hosting and maintenance to the local Belgian company Kinamo.

During this phase, we will relocate from the Biosensors group to the Leuven Bioincubator as a spin-off company associated with KU Leuven. We will also reach out to more venture capital firms to obtain seed funding to keep our team afloat while developing our base of sales in the Benelux region. Due to the labour costs associated with chip manufacturing, we will outsource the chip-making to Schott Minifab or Micronit, companies specialised in microfluidic chip design for life sciences. However, we will avoid a dedicated product warehouse to save costs. We will instead use low-cost storage lockers to keep the cartridges before shipping. To ensure customer satisfaction, we will have a customer service team keep in close contact with our current device customers and allow them to receive technical advice from our engineers in case of device errors or if they would like additional features.

Phase III: Expanding sales: In this phase, **we intend to expand our base of sales to the broader EU market** by cooperating with Benetec, which is a local medical device distributor. In addition to marketing our device as a CT screening tool, we will also optimise our product as a long-term monitoring tool for TBI based upon the data collected (long-term data on biomarkers and patient wellness) through the app in the previous phase collect upon opt-in consent by users for research and marketing. Data collected from these consenting users will be marketed to research institutions in Europe and the US for data validation of chronic TBI and rehabilitation.

Phase IV: Expand portfolio: Once we establish stable profitability, an expansion of our market to other countries such as the US, Japan, and later LMICs will be the next step. Patent assignments will be done according to market destination (i.e. USPTO and JPO). Moreover, resources will also be devoted to R&D for further improvements of our product and to seek new possibilities of biomarkers.

5.5 Financial Viability

To provide concrete support for our business plan, we compiled a more comprehensive overview of the finances involved. (Figure 9, Appendix 9.8-9.10). We constructed this plan with the help of advisors such as Francesco Dal

Dosso. The ideas discussed throughout this TRD have also been supported with TBI experts from the TBI research centre in Antwerp, CENTRE-TBI. Our predicted financial status over the three phases is summarised in Figure 9 and in Appendix 9.11. The revenue stream will comprise out of selling: (i) our readout devices, the Pulse EISY, which will retail for €3000, (ii) the microfluidic cartridge used to perform the TBI test for each patient individually, which will retail for €40 per chip, and (iii) the sale of curated data from (obliging) users. A key metric to our company's success is the continued sales of cartridge to customers who have bought the read-out devices. Although we make limited profit on our device (€1966.55), we are pursuing a razor blade model, where we make the bulk of our income through the profit on cartridges sold.

For this, we will aim to achieve high customer satisfaction by (i) keeping close relations with customers (ii) and closely collaborating with our customers on pathways for improving device (primarily in phase I). Although we will eventually target the greater European market, sales will first be established in the Benelux region. There are an estimated 799 hospitals spread amongst the three countries and an estimated 60000 cases of TBI per year in the Benelux area (Michas, 2022b, 2022a; Sarita, 2023). We aim to reach 5% of this market by the end of phase II, which will translate to the sale of the Pulse EISY readout system to approximately 40 hospitals and the sale of 2750 TBI cartridges per year. This amounts to €230000 per year in cartridge and device sales.

Once we have established a solid base of sales in the Benelux region, we will move to targeting the greater European market, which has a TBI incidence rate of 258 per hundred thousand and a population of 746.4 million people (Maas *et al*., 2017). With 2% of the 5000 that use the Pulse EISY, a revenue stream of more than €2 million can be realised at the end of phase 3 excluding the revenue from the mobile application.

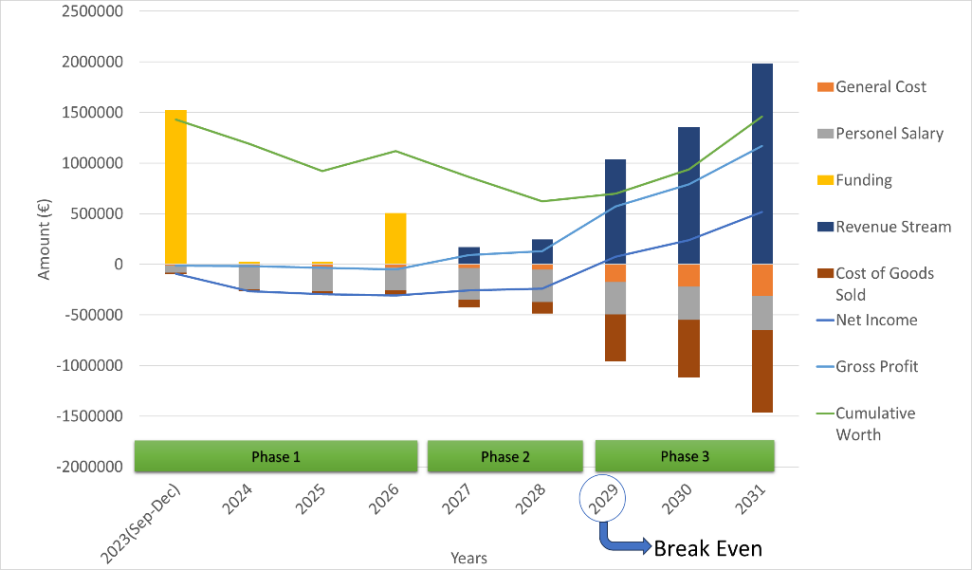


***Figure 9: Financial Summary of our company over the coming 8 years*.**

To be successful, a significant investment is required until we are able to gain enough market share to become stably profitable. We will initially court grants from the Eurostars funding program, Gemma Fiscus, VLAIO, and other grants close to home. Once we are able to form a spin-off company from the Bioincubator, we will begin courting venture capital groups to support our operation. From these sources, we hope to obtain €1.5 million to keep us afloat over Phase I, where we are not making a positive net income.

6. Team and support

6.1 Contribution of team members

Emily Brugger is team captain of the bioassay team and communication responsible. As team captain, she single handedly supported her team through the development and optimisation process of the bioassay. Additionally, she maintained our social media platforms.
Anton De Brabandere is a member of the technology team. His main focus was on the management and (pre)processing of all the CV and EIS data produced over the course of the competition.
Lander De Waele is a member of the technology team. He helped a lot with the design and practical part of the cartridge design. He also contributed to the development of electrochemical readout, box design, and electronic design. He made the phone application and established the cloud connection. At the innovation days he will present our results during the technical pitch.
Lotte Van Landschoot is a member of the bioassay and business team. Over the whole course of the competition she remained a fixed value in the lab, tinkering on both the bioassay and business development.
Martin Van Wymeersch is a member of the technology and business team. He managed the majority of the electronics design personally.
Robert Forsyth is a member of the technology and business team. In addition to overseeing the majority of the software and GUI development, he personally handled the 3D visualizations of the box. As part of the business team, he will also be presenting the translational potential pitch.
Stef Cuyckens is a member of the readout team. He managed the majority of the box design personally and was partially responsible for researching the components.
Shaishav Shah is a member of the technology and business team. He split his time between assisting with the cartridge fabrication and the business proposal.
Truong Giang is a member of the technology team. He assisted in the printing, assembling, and testing of the cartridge.
Thibaud Werpin is a member of the bioassay team. During the competition he was a major contributor in the lab, performing the bioassays and exploring potential avenues for improvement.
Stephanie Tse is team captain of the technology team and member of the business team. As team captain, she guided her team through the whole process of cartridge development.

6.2 Additional support

Our first word of gratitude goes out to prof. **Jeroen Lammertyn**, as founder of the biosensor MeBios group at KU Leuven, he provided us with the needed resources, equipment, and support.

Secondly, we wish to dedicate a section to our four coaches, who have played a pivotal role in our journey. As PhD candidates within the MeBios biosensor group they supported us throughout the past nine months. Their expertise and dedication helped us not only in realising the device, but also encouraging a good team cohesion. **Emiel De Rieck** contributed significantly to the realization of the bioassay, **Francesca Pollet** focused her efforts on the cartridge design, **Claudia Scarpellini** adeptly coordinated technology and read-out aspects, while **Julie Van Lent** played a vital role in crafting a robust business case. Furthermore, we would like to dedicate some words to thank **Dries Vloemans** and **Lorenz Van Hileghem** with their years of experience they showed to be a vital support on the (i)SIMPLE design, **Lieze Dankers** for her support in surface functionalisation strategies, **Karen Leirs** for both her valuable insight and advice and **Francesco Dal Dosso** for helping with the business part and providing us with feedback.

Last but not least, we extend a special acknowledgment to **PalmSens** for their critical support in interpreting and processing EIS data. Their tools have been instrumental in refining both the EIS and molecular recognition design.

6.3 Sponsors

MethrOhm emerges as a significant partner, providing not only financial support but also its expertise on electrochemistry that proved to be indispensable to our project.

7. Final remarks

The team PULSe hopes this document convinced you of the potential that arises from combining electrochemical techniques, such as electrochemical impedance spectroscopy, and the (i)SIMPLE technology within a POC framework. With the initial prototype progressing steadily, our future endeavours aim to delve deeper into exploring the device's capabilities, particularly in terms of LoD and sensitivity parameters. Additionally, the incorporation of a Bluetooth link to a smartphone, along with its corresponding application, holds the promise of extending the device's utility to resource-constrained environments, such as rehabilitation institutions. This augmentation has the potential to not only mitigate fabrication costs but also streamline the device's functionality by eliminating the need for a screen.

Over the past nine months, we have learned an entire portfolio of skills, going from technical and business aspects, but also some soft skills such as communication and interdisciplinary teamwork. As a final note, we want to thank everyone who supported our team over the course of this journey. We also wish to express our sincere appreciation to the SensUs organisation for orchestrating this remarkable competition. We are eagerly looking forward to meeting all teams at the innovation days in Eindhoven.

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9. Appendix

9.1 Incubation cell

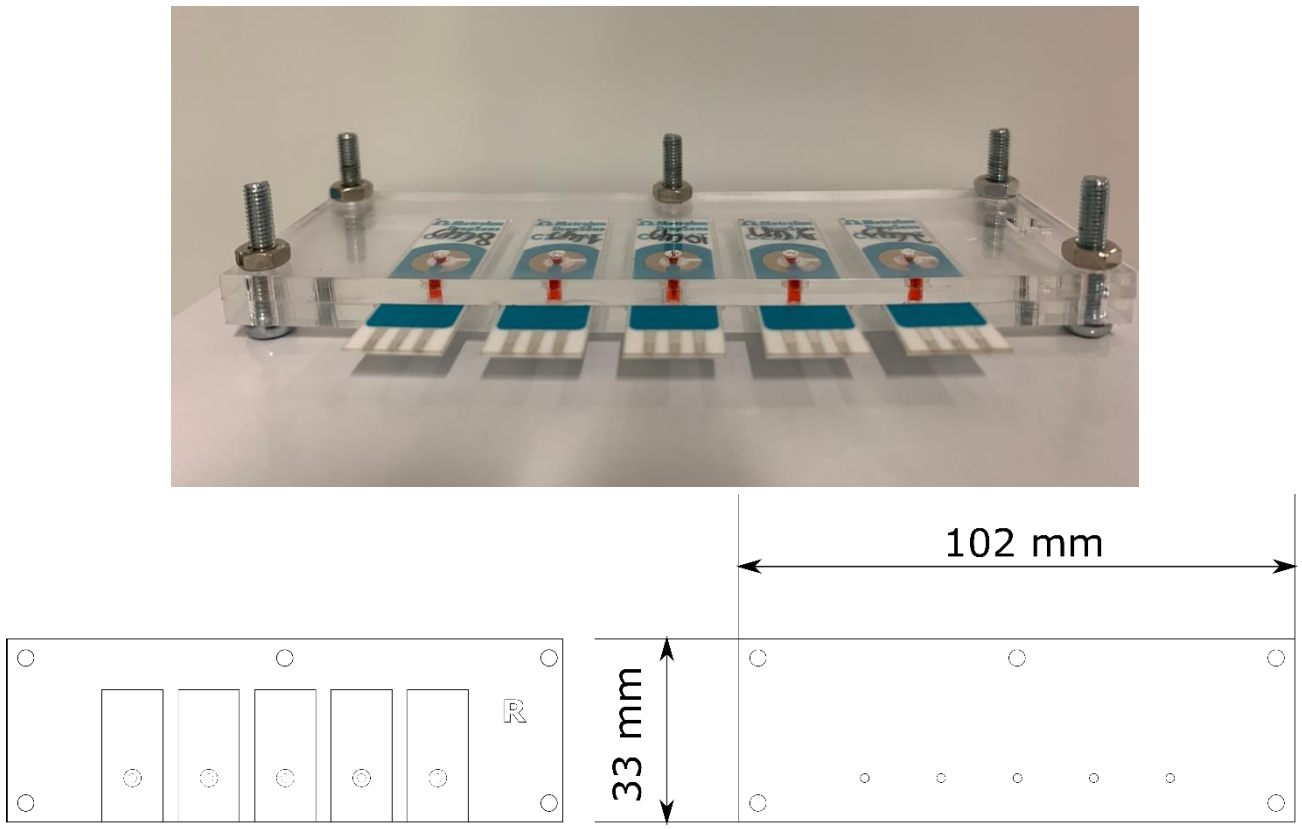

Figure 10: picture and design of the incubation cell. The cell is used for the incubation of functionalised AuNP. The cell is made from PMMA. Around the WE, there is a sticker made of PSA that sticks to the electrode and the PMMA to prevent leaking. At the end, the screws are tightened to assure a leak free incubation chamber.

9.2 Cartridge flow

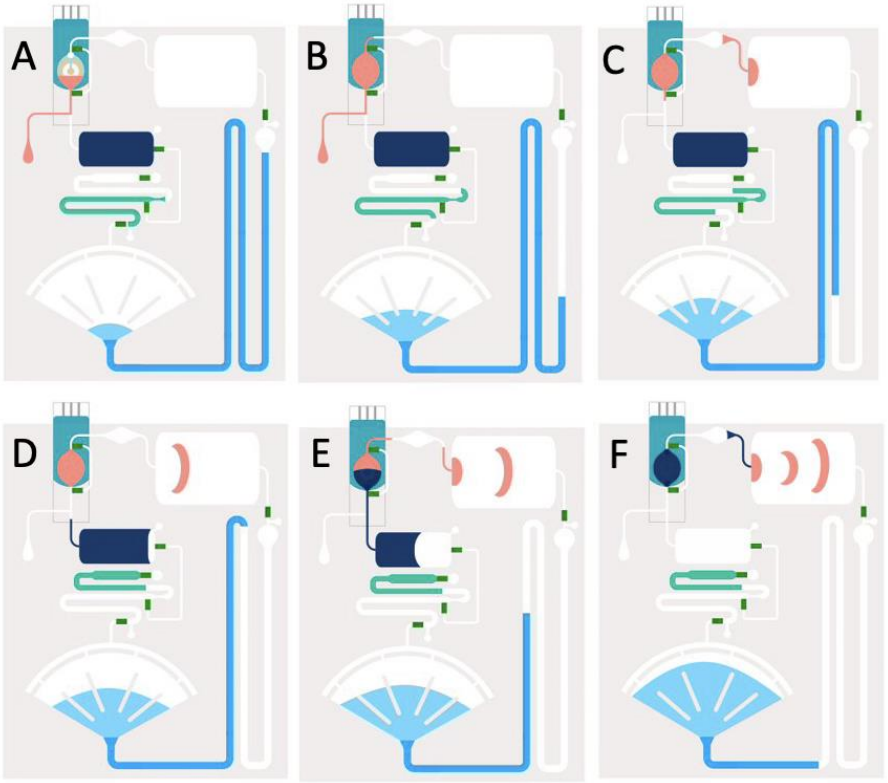

Figure 12: Schematic diagram of cartridge flow steps. This diagram displays the time course of liquid flow inside the cartridge (***A – F***). **A)** The pump is activated, pulling sample liquid (red) into the SPE incubation chamber and pushing the timer (green). **B)** Consecutively the sample liquid exits the SPE incubation chamber approaching the LED detection chamber. **C)** Following this the sample liquid incubates inside the SPE incubation chamber, extra waste liquid flows through the LED detection chamber into the waste chamber. **D)** Next, the timer reaches a barrier which initiates the pump to start pushing the redox-couple (dark blue) out of the reservoir. **E)** This is followed by the sample liquid being washed away by the redox-couple. **F)** Lastly, the redox-couple incubates in the SPE incubation chamber; left-over redox-couple leaves the LED detection chamber, triggering the SPE detection and corresponding algorithm.

9.3 Nyquist plots

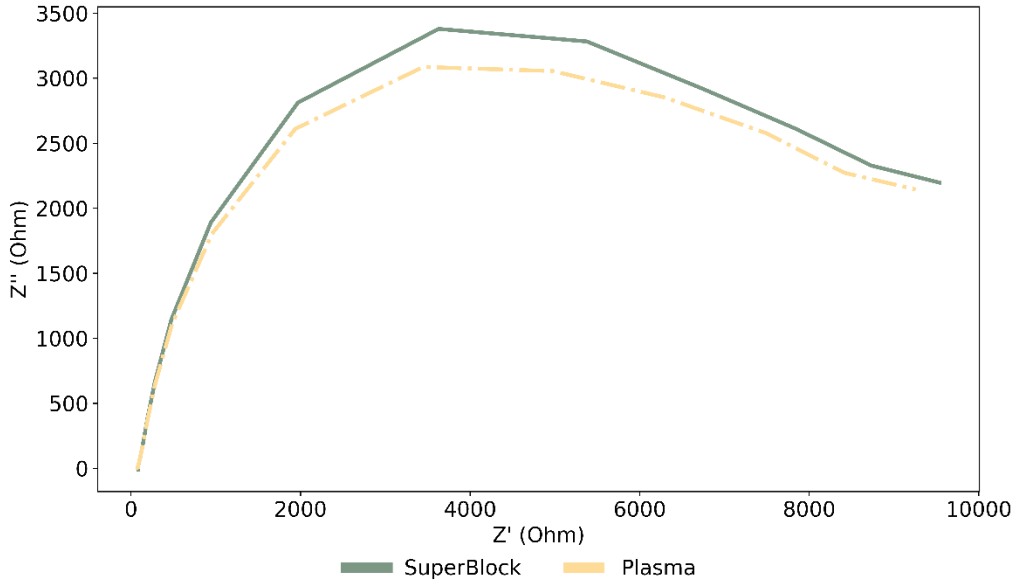

Figure 11: EIS graph showing resistance difference after plasma incubation. *The fitted* R_{CT} values have a difference of around 239 Ohms.

9.4 Dye dilution

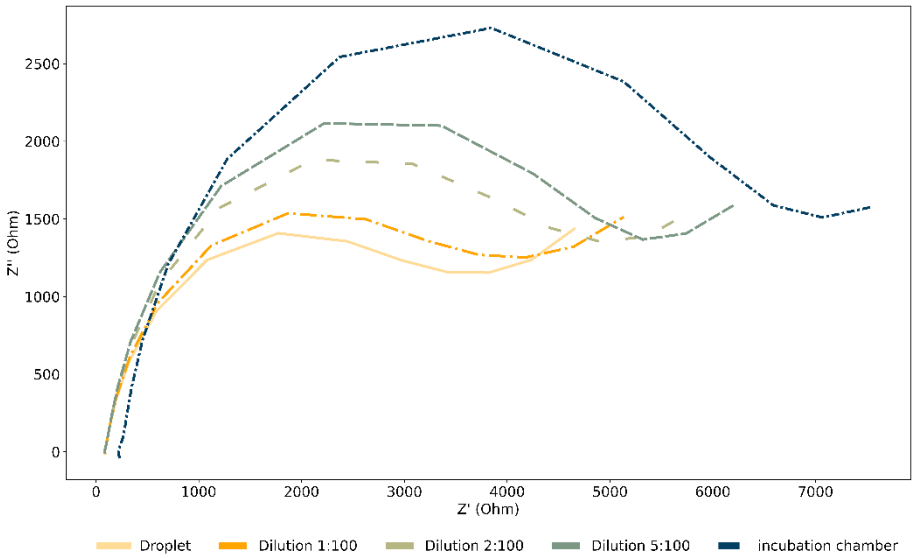

Figure 13: EIS graph showing difference in EIS signal between different colour dye dilutions and between droplet and incubation chambers.

Table 1: Difference in solution resistance (R_S) between different colour dye dilutions and between droplet and incubation chambers.

	Normal	Dilution 1:100	Dilution 2:100	Dilution 5:100	Chamber
Repeat 1	86.74	84.79	86.88	87.72	228.5
Repeat 2	87.93	84.35	84.51	86.53	224.2
Repeat 3	88.58	86.83	88.35	88.13	232.6
Mean	87.75	85.32	86.58	87.46	228.43
Standard deviation	0.93	1.32	1.93	0.83	4.20

Table 2: Difference in charge transfer resistance (R_{CT}) between different colour dye dilutions and between droplet and incubation chamber.

	Normal	Dilution 1:100	Dilution 2:100	Dilution 5:100	Chamber
Repeat 1	3221	3286	4189	5215	6456
Repeat 2	2620	3097	4191	4780	6257
Repeat 3	4030	4621	5081	5041	6496
Mean	3290.33	3668	4487	5012	6403
Standard deviation	707.55	830.71	514.42	218.94	128.01

9.5 Incubation and detection

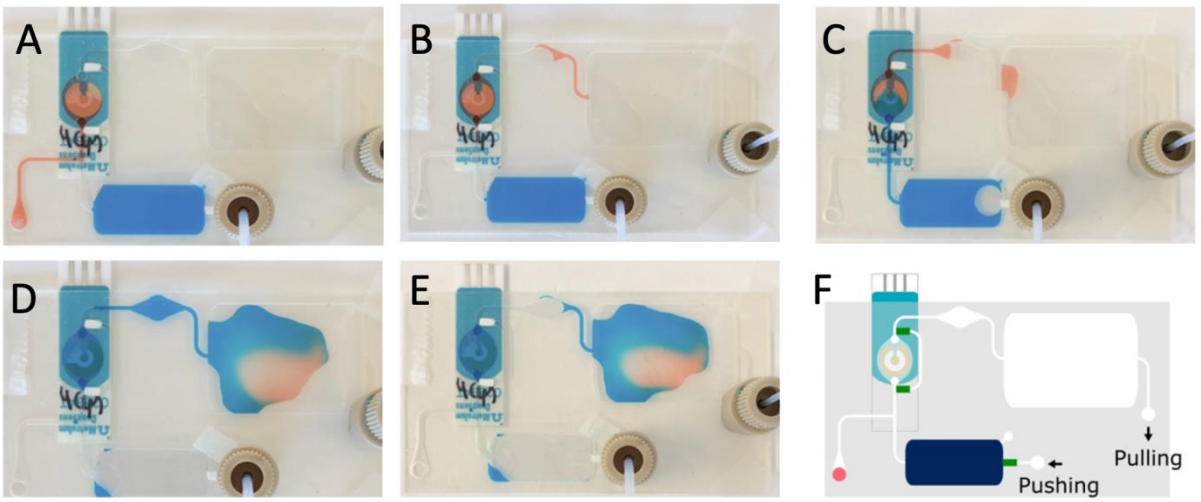

Figure 14: Real-life demonstration of steps of incubation and infrared (IR) light between the LED and the phototransistor. These photos show the isolated incubation and detection systems, powered by a Harvard Apparatus Syringe Pump, to illustrate the main steps ***(A – E)*** of the incubation inside the SPE incubation chamber and the activation of the detection algorithm at the LED detection chamber, where the diagram***(F)*** is a representation of the isolation design. **A)** To begin, the sample liquid (red) pulled towards the incubation chamber, **B)** Then, as sample liquid incubates inside the SPE incubation chamber, extra liquid exits via the LED detection chamber to the waste **C)** Subsequently, the redox-couple (blue) enters the SPE incubation chamber, washing the sample liquid out, **D)** Redox-couple hence incubates on top of the electrode instead, while left-over liquid exits towards to the waste, **E)** Finally, the left-over redox-couple leaves the LED detection chamber, detection algorithm triggered for SPE detection.

9.6 QR code scanner

Figure 14: Graphical representation of the 2D barcode scanner, mounted on the side of the reader device above the cartridge. *This is used to transfer chip specific calibration data to the device, which is needed to calculate the ΔR_{CT} accurately.*

9.7 Box design and dimensions

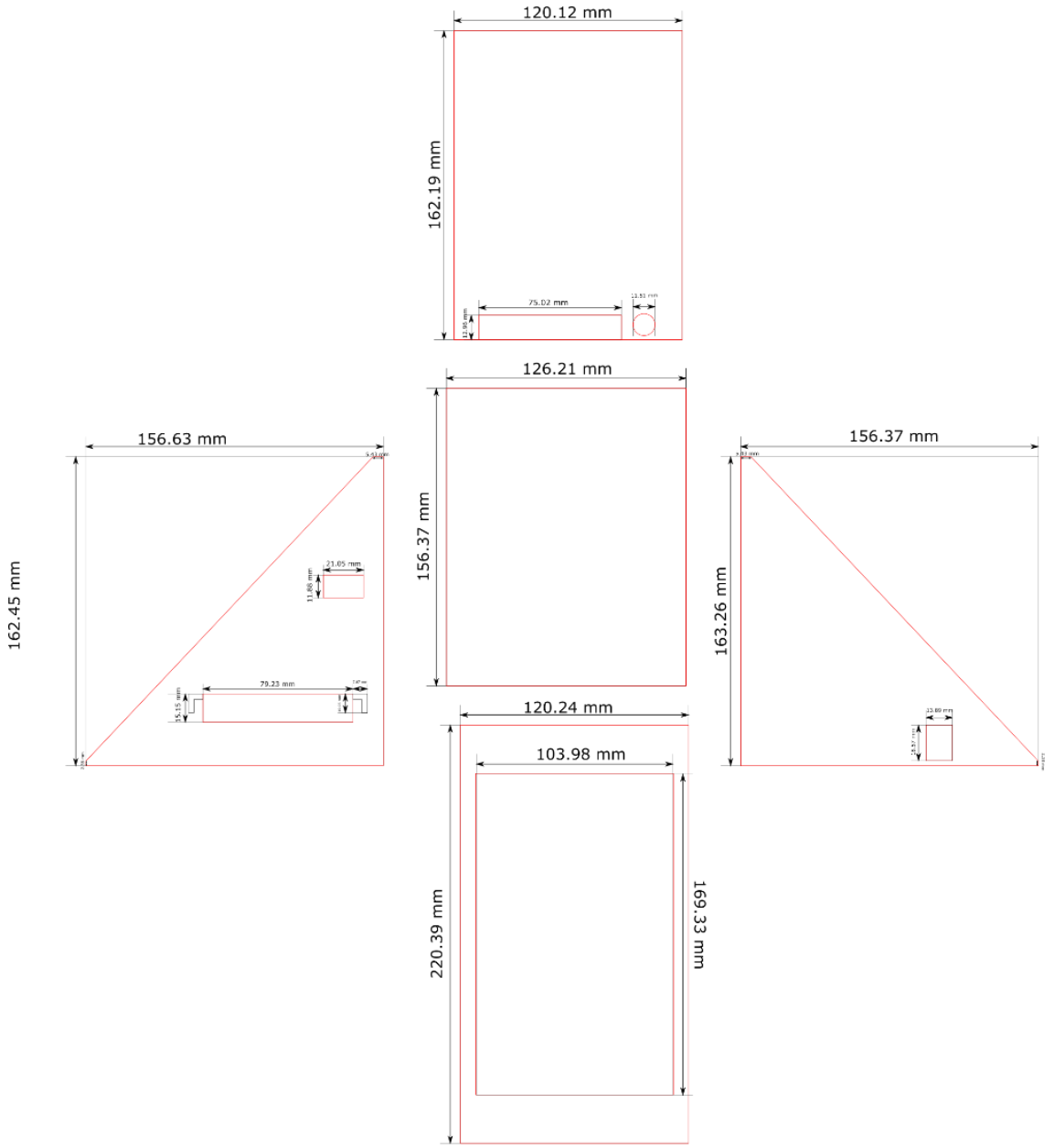

Figure 15: Dimensions of the reader device.

9.8 Assumption and Reasoning Used During Calculations

Below some sections of the business calculations have been explained in detail. Some things should be kept in mind for the calculations of the personnel salary and funding. The personnel salary was simply calculated by looking at what the normal rates are for each position in Belgium this value was then used along with a 2% increase year on year.

For the funding various sources were considered, the first being Eurostars, this is a non-dilutive funding option which is available in Flanders (and in other member countries). They will fund up to 50% of your project cost. However, you need to prove that you have sufficient funds to continue your work without them (Michiels & Menten, n.d.). The next funding option is the Gemma Frisius Fund, this fund is set up by KU Leuven to aid startups. They do, however, take control of a certain portion of the company after they invest. This funding period normally continues for up to 7 years (KU Leuven, 2022). The other sources of funding are merely from family, friends and companies who are interested in our product.

9.9 Revenue Stream

As stated in the document the revenue stream consists of the cartridge sales, Pulse EISY sales and finally the data access subscriptions. To determine the amount of revenue generated by each product, research was carried out and some assumptions were made. The details will be specified below.

A method used to calculate the revenue stream for the chips and Pulse EISY, was to determine the number of sales which seemed reasonably achievable. This amount was set as the goal for the end of a specific phase. In the years leading up to this goal, sales are slowly ramped up to the desired amount. The numbers below highlight the different benchmarks used for each calculation.

For the subscription service, a different approach was used. It was estimated that a mere ten percent of customers will make use of the application after being treated (tested for TBI). This can then be included in the calculation by summing the total number of tests taken and then calculating ten percent of this value. We valued each active user at about €15. This value was chosen by keeping in mind the potential value the data could bring. Today active users' medical history is easily sold for €35 (Bergemann *et al*., 2020). To keep the subscription price up-to-date, we recalculated the subscription cost at the beginning of each phase and then use that data for each of the years in that phase.

Phase 1:

- There will be no income generated as we will be carrying out research.

Phase 2:

- 55 000 TBI cases in Benelux (Abbott Laboratories, 2011; Reger *et al*., 2022)
- 799 hospitals in Benelux (Abbott Laboratories, 2011; Reger *et al*., 2022)
- €3000 per Pulse EISY
- €40 per chip
- Capturing 5% per cent of the market tallies to = €230000 by 2028 for the cartridges and the readout devices.
- Selling data to companies UZ Leuven and CENTRE-TBI summing to €15450 year on year.

Phase 3:

- 15000 hospitals in the EU and 746.4 million people in the EU with a crude TBI rate of 258 per thousand per year results in = 1925712 cases (Brazinova *et al*., 2021).
- We tackle 2% of this market for the sale of our devices and chips and we assume that only 50% of cases are tested. This is a normal percentage of mild TBI cases in relation to other forms of TBI. This sums to about 19257 chips and 300 devices by the end of 2030. Adding this altogether a value of (19257 * 40) + (300 * 3000) = €1670 280 can be expected at the end of 2031.
- By the end of phase three it is expected that about ten organisations will be paying for access to our data. This increase is relatively realistic as we have by this point gained significant market exposure. Our subscription service is set-valued at €2090 per year. This totals to €313500 by the end of phase 3.

9.10 Costing

Below you can find a more detailed estimation of the cost of goods of the Pulse EISY device and the cartridges. The material cost was calculated with the numbers we have available to us. The manufacturing of each respective device was estimated using market research. To do the costing of the application to track and aid patients was done by calculating how much it cost to rent a server and keep the application available on the app store for both the Play Store and the Apple Store. The cost of development and maintenance was included in the salary of the computer

scientist hired (this cost is not shown).

The cost of goods was kept constant throughout the years. This is a conservative estimate because our product will likely become less expensive. The cost of the hosting servers was increased in the third phase as we would be dealing with larger amounts of data.

We used the data in Table 3 to determine a cost of €1040.95 per sensor and € 31 per chip

Table 3: Sensor Material Cost Overview

Material	Price(€)
SparkFun 2D Barcode Scanner Breakout	53.5
Raspberry PI 2 Model 3B Version 2	49.95
Photo-Resistor Circuit	5
Power Supply	15
LCD Screen	45
USB Expansion Hub	15
Em-Stat PICO Development Kit	850
Total	1033.45

Table 4: Chip Material Cost Overview

Material	Price(€)
Chip Material	8
Metrohm dropsense c223at	3
Total	11

Table 5: Overview of Chip Assembly Costs

Assembly Requirement	Cost (€)
Chip Manufacturing Cost(€/Chip)	15
Estimated time to assemble sensor(h)	0.5
Cost of a technician in Belgium(€/h)	35

9.11 General Cost

The general costs will be discussed in more detail in the following section. Each of the costs will be discussed separately.

- Infrastructure, when the spinoff is created office space will need to be rented which is about €400 per month in Leuven, an extra €2500 has been added for money for stationary and other costs regarding running an office (Bean-Mellinger, 2019).

- Intellectual property registration and defence costs are about €100000. This value is rather speculative, but an approximation was made considering that a patent cost between €10000 and 15000 to reach the granting, and then maintenance feed need to be paid in the regions selected (FOD economie, 2023). The value can easily be exceeded if a legal battle takes place.

- R&D costs will be covered by the university as we will be making use of their resources. The salaries of our employees have been included in previous calculations. Once we reach the market, we will make use 10% of our revenue to carry out research and development.

- Accreditation refers to the process that needs to be followed and the steps that need to be paid for and taken to have our device accredited. The specifics can be seen below:

  - Price of accreditation €9479.90 for a Class 1 or 2 device (FAMHP, 2021).

  - An expert will also be consulted to advise with the accreditation. (€20000- a normal fee for consultants)

- Marketing and Sales, about 5% a year of revenue is used to fund the advertising.

9.12 Calculation summary

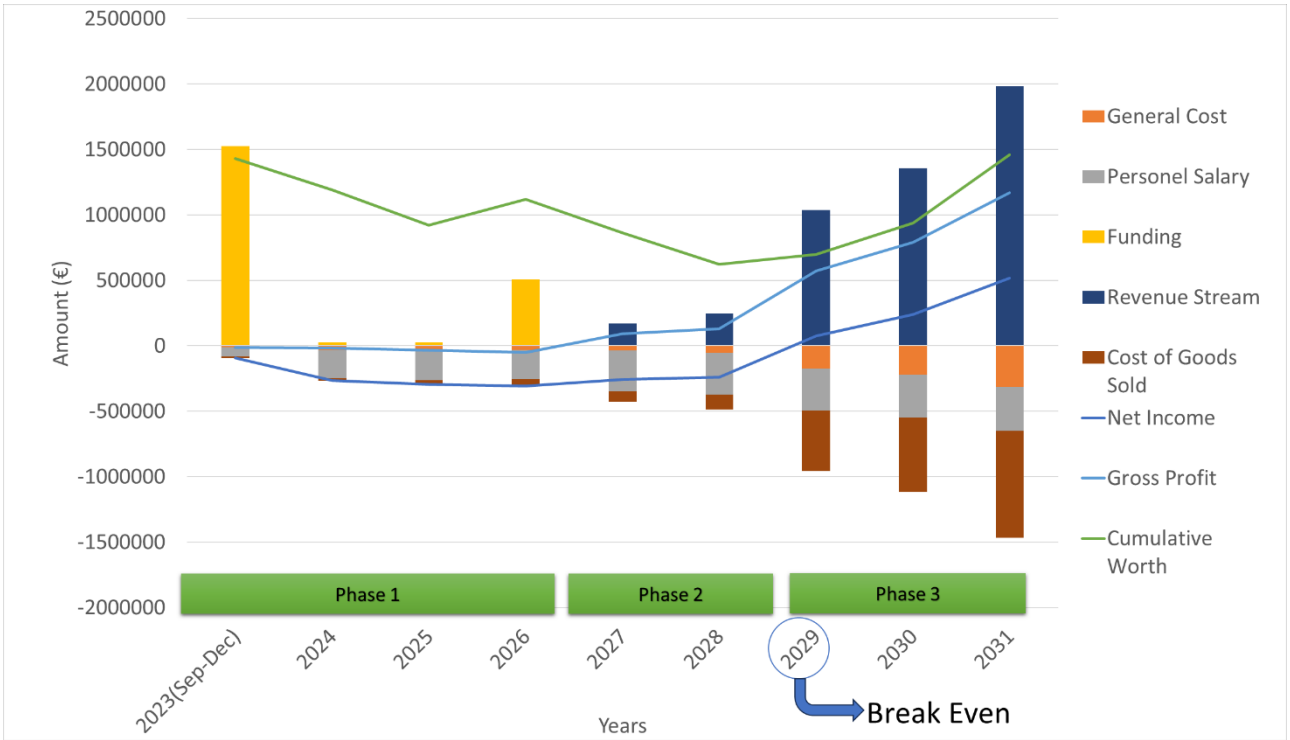

Figure 16: Summary of Costs and Revenue over time marked with our business plan phase structure. This graph summarises the costs and revenue estimation over the next decade (from phase 1 to phase 3: (1)test phase and initial sales, (2) selling in local Benelux market, (3) sales in the European market). Expenditures include general cost from starting up, salaries of employees, and manufacturing costs. Fundings and grants will make our initial financial support, and an increasing revenue stream will be seen as we enter the local market. A break even is anticipated by 2029.

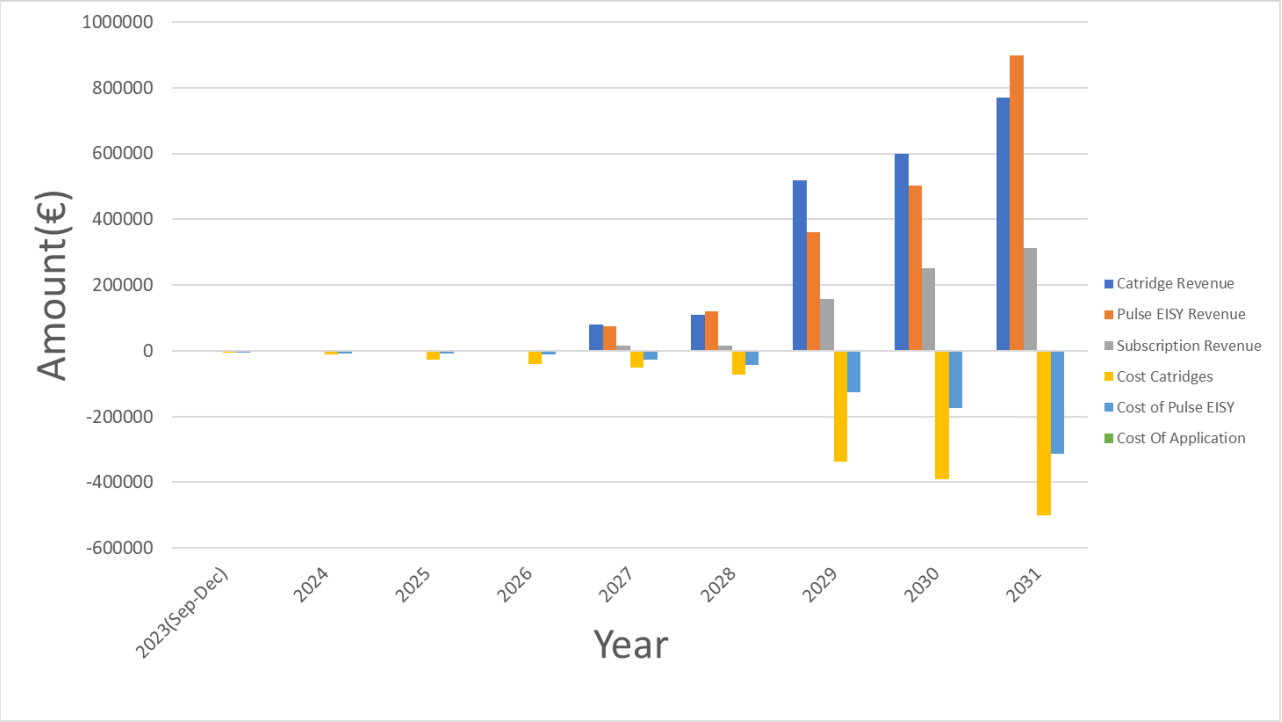

Figure 17: Summary of Revenue and Cost of goods over time. The graph shows the estimated total annual revenue and costs from 2023 to 2031, with the revenues and costs of the cartridges, PULSe EISY, Data subscription taken into account.

Phase		Start Up	Testing Phase, and initial sales	Testing Phase, and initial sales	Testing Phase, and initial sales	Selling in Local Benelux Market	Selling in Local Benelux Market	Moving Sales to larger European Market	Moving Sales to larger European Market	Moving Sales to larger European Market
Revenue Stream (€)		**0**	**0**	**0**	**0**	**170450**	**245450**	**1036750**	**1354800**	**1983780**
PULse Chip	Unit Price	0	0	0	0	40	40	40	40	40
	Number of Units	250	400	1000	1500	2000	2750	13000	15000	19257
	Total Units Sold	250	650	1650	3150	5150	7900	20900	35900	55157
	Revenue	0	0	0	0	80000	110000	520000	600000	770280
Pulse Sensor	Unit Price	0	0	0	0	3000	3000	3000	3000	3000
	Number Of Units	5	7	7	10	25	40	120	168	300
	Total Units Sold	5	12	19	29	54	94	214	382	682
	Revenue	0	0	0	0	75000	120000	360000	504000	900000
Access To Database	Total Data Points	25	65	165	315	515	515	2090	2090	2090
	Number of buyers	0	0	0	0	2	2	5	8	10
	Cost Per Data Point	15	15	15	15	15	15	15	15	15
	Revenue	0	0	0	0	15450	15450	156750	250800	313500
Cost Of Goods Sold(€)		**- 12,068.75 €**	**- 18,025.65 €**	**- 33,625.65 €**	**- 49,748.50 €**	**- 78,362.75 €**	**- 113,477.00 €**	**- 463,493.00 €**	**- 565,458.60 €**	**- 813,546.00 €**
Chip Material	Unit Cost	11	11	11	11	11	11	11	11	11
Chip Manufacturing	Unit Cost	15	15	15	15	15	15	15	15	15
	Number of Units	250	400	1000	1500	2000	2750	13000	15000	19257
	Cost Of Good	-6500	-10400	-26000	-39000	-52000	-71500	-338000	-390000	-500682
Measurement Unit Cost	Unit Cost	1033.45	1033.45	1033.45	1033.45	1033.45	1033.45	1033.45	1033.45	1033.45
Measurement Unit Manufacturing Cost	Unit Cost	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
	Number of Units	5	7	7	10	25	40	120	168	300
	Cost Of Good	-5204.75	-7286.65	-7286.65	-10409.5	-26023.75	-41638	-124914	-174879.6	-312285
Associated Server Costs		240	240	240	240	240	240	480	480	480
App Store costs		124	99	99	99	99	99	99	99	99
Total App Cost		-364	-339	-339	-339	-339	-339	-579	-579	-579
Gross Margin(€)		**-12068.75**	**-18025.65**	**-33625.65**	**-49748.5**	**92087.25**	**131973**	**573257**	**789341.4**	**1170234**
Funding (€)		1525000	25000	25000	505000	0	0	0	0	0
Eurostar Funding		25000	25000	25000	25000					
Gemma Friscus Fund					400000					
VLAIO					50000					
Collaborators					20000					
Family and Friends					10000					
Private Investors		1500000								
Personnel Salary Before Deductions(€)		**-360000.00**	**-360000.00**	**-367200.00**	**-374544.00**	**-522034.88**	**-532475.58**	**-543125.09**	**-553987.59**	**-565067.34**
(At ist largest the team will consist of 6 Employees with the Engineering Lead acting as a CEO)										
Engineering Team Lead(Phd)		100000.00	100000	102000	104040	106120.80	108243.22	110408.08	112616.24	114868.57
Biomedical Engineer(Phd)		85000	85000	86700	88434	90202.68	92006.73	93846.87	95723.81	97638.28
Computer Scientist		85000.00	85000	86700	88434	90202.68	92006.73	93846.87	95723.81	97638.28
Finance and Legal Advisor		90000	90000	91800	93636	95508.72	97418.89	99367.27	101354.62	103381.71
Sales and Marketing		0	0	0	0	70000	71400.00	72828.00	74284.56	75770.25
Adminstration		0	0	0	0	70000	71400.00	72828.00	74284.56	75770.25
General Costs(€)		-10000	-30000	-39479.9	-30000	-37754	-54117.5	-172812.5	-220520	-314867
Infrastructure		0	0	0	0	7300	7300	7300	7300	7300
Intellectual Property(Creating, Defending and Registering)		10000	10000	10000	10000	10000	10000.00	10000.00	10000.00	10000.00
Research Costs		0	0	0	0	17045	24545	103675	135480	198378
Acreditation		0	20000	29479.9	20000	0	0	0	0	0
Marketing and Sales		0	0	0	0	3409	12272.5	51837.5	67740	99189
Net Income		-382068.75	-408025.65	-440305.55	-454292.50	-467701.63	-454620.08	-142680.59	14833.81	290299.66
Cumulative Wealth		1142931.25	759905.60	344600.05	395307.55	-72394.08	-527014.16	-669694.75	-654860.94	-364561.28

Figure 18: An Excel spreadsheet summarising the sources of revenue, costs, and funding.