

Team Results Document

UppSense



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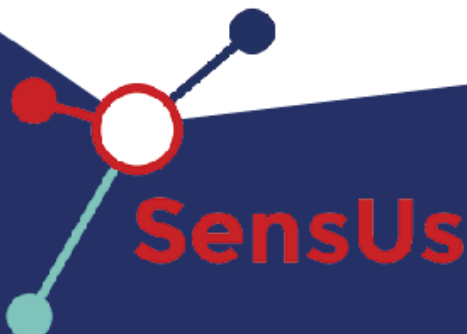
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12/08/2022



UPPSALA
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SensUs 2022

Acute Inflammation with a focus on sepsis

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1. Abstract

Sepsis is a life-threatening condition where the host's immune system overreacts to an infection, causing damage to self-tissues and organs. Interleukin-6 (IL-6) is a cytokine that is present in the bloodstream, and its concentration increases substantially in sepsis. For rapid diagnosis of sepsis, UppSense team has developed an electrochemical-aptamer (DNA strand) based sensor suitable for measuring IL-6 concentration from 1-1600 pg/mL in blood plasma within 10 minutes. The IL-6 specific aptamer is bound to a gold electrode. The cartridge we designed is of low cost (manufacturing cost € 0.92), easy to manufacture, and holds 20 µL of plasma sample. Upon binding to IL-6, the aptamer changes conformation, and we measure IL-6 concentration with the electrochemical impedance spectroscopy. Such application of electrochemical impedance spectroscopy in IL-6 electronic aptamer-based sensor has never been described before. The results we get in an user-friendly app designed by the team. We have developed business model for the commercialization of biosensors. Our business model aims to achieve € 1.390.000 revenue by the year 2032. With great enthusiasm, we are presenting sensitive, specific, cost-effective, and user-friendly biosensor for rapid detection of IL-6.

2. Biosensor system and assay

The biosensor is based on the technology of electrochemical aptamer-based (EAB) sensors¹. This class of sensors is able to detect and quantify analytes based on the change of conformation that the oligonucleotides undergo upon binding. Specifically, this is possible to detect by decorating an electrode with an aptamer functionalized with a redox tag. Hence, electrochemical techniques are used to observe this phenomenon.

2.1 Biorecognition element

The biorecognition element is an IL-6-specific-aptamer selected after literature survey^{2,3}. To identify the best performing aptamer, the kinetics and affinities were determined using surface plasmon resonance (SPR). The kinetics are also under investigation using grating coupled interferometry (GCI) in order to further confirm the parameters established with SPR. Furthermore, it is going to be determined how these parameters change when varying plasma percentages are introduced into the buffer.

2.2 Signal transduction

The selected aptamer is then functionalized with methylene blue, acting as redox tag, on one end and a thiol C6 linker on the other end, in order to immobilize the DNA strand on gold electrode. Upon binding to the protein, the oligonucleotide undergoes a change in conformation that changes the equilibrium position of methylene blue (Figure 1); thus, the electron transfer process from the redox centre to the electrode surface is affected. In particular, this can be detected with electronic impedance spectroscopy (EIS)⁴, as a change in the phase shift, using a three electrode set-up.

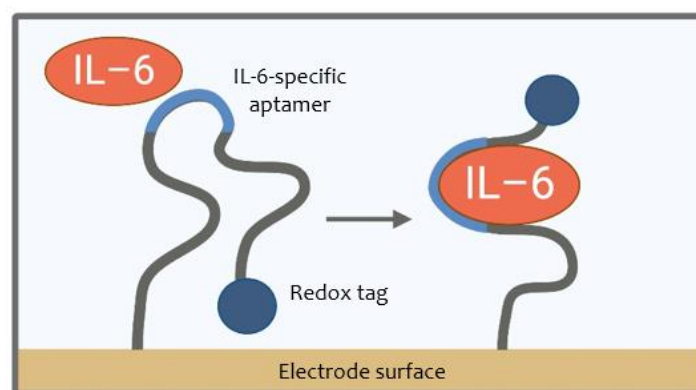


Figure 1: Schematic representation of the mechanism of an EAB sensor. The blue sphere is methylene blue, while the green one is the analyte (picture made via Biorender).

2.3 Cartridge technology

The biosensor was thought to be as simple and cheap as possible to keep it affordable and easy to use. Thus, the sample holder is a well, created by additive manufacturing. The sample is pipetted in the well at the bottom of which are positioned the three electrodes. The printed wells are coated with bovine serum albumin to prevent the aptamers from adhering on their surface during the functionalisation of the gold electrode. The wells are glued on a glass plate using silicone glue to seal the passing-holes of the electrodes and to insulate the wires sticking out of the wells.

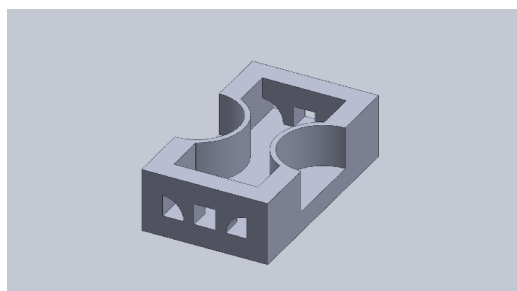


Figure 2: 3D printed well for the three wire-electrodes (silver CE, functionalized gold WE, Ag/AgCl RE).

The cartridge is then directly connected by the wires to the potentiostat using an adaptor. The potentiostat is fixed in an aluminium faraday cage which in turn is placed in a 3D printed casing.

2.4 Reader instrument

The reader instrument is composed of three elements: EmStat Pico Potentiostat, Raspberry Pi 3 and Raspberry Pi 7 inch touch screen. The ease of use of these components, together with their compactness, portability and affordability makes this the most suitable choice for our biosensor. The Raspberry Pi touch screen represents the user-interface of the device.

The total size for the biosensor is 110 mm x 85 mm x 65mm for the potentiostat and its casing and 170 mm x 110 mm x 60 mm for the Raspberry Pi (Figure 3).

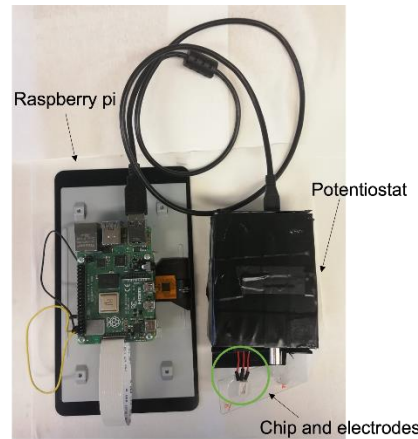


Figure 3: Electronic set-up of the sensor.

2.5 User interaction

The biosensor is fully automatized. Data is directly taken from the Palmsens software and analysed through a script that outputs the calculated IL-6 concentration (pg/mL) and displays it on the screen.

The case when the concentration is below or above the detection limit is also considered as it could be very relevant, especially in the case when the concentration is too high. Concentration is classified into one of the five available categories (healthy, inflammation stage 1, inflammation stage 2, sepsis, septic shock), which are marked with a colour code (from green to red) (Figure 4)

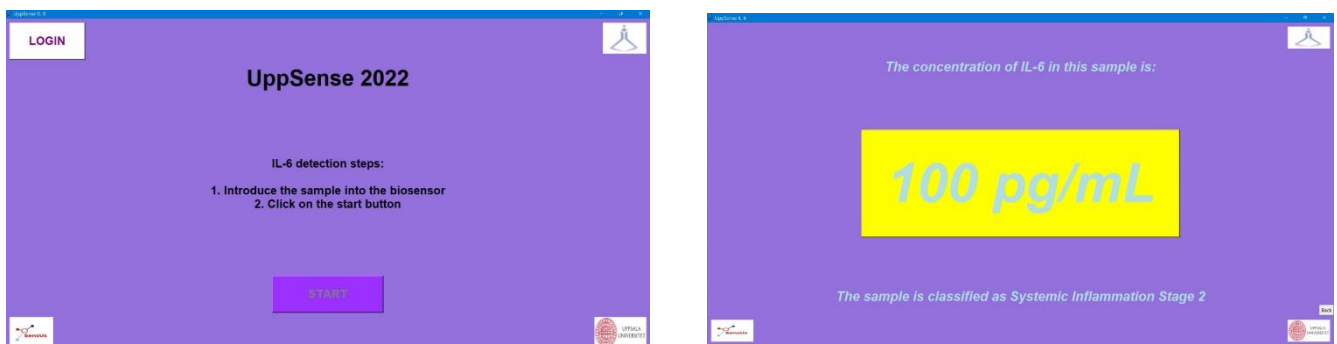


Figure 4: Software home page (left) and example of results output (right).

The app was developed in a simple way, to provide the user with an user-friendly biosensor and to minimise the direct interaction with the sample.

The possibility of registration and login is thought mostly for hospital applications or for users who want to keep track of the different performed measurements. The user profile contains relevant information about the patient and a history of all the previous measurements (date of the measurement and relative result). Moreover, there is the possibility to download the patient history in an Excel format. This allows the use of the obtained data in further analysis and tests. Taking single measurements without login remains available to facilitate single time users and acute cases.

3. Technological feasibility

3.1 Biorecognition element

Three aptamer sequences from literature were screened for binding to IL-6 with SPR, one 16-nucleotide (NT)², one 31-nucleotide² and one 57-nucleotide³ aptamer. All SPR analysis was performed with the Biacore 8K instrument using single cycle kinetics⁵ (SCK), otherwise known as kinetic titration. Initially the aptamers were immobilized onto the sensor chips through a biotin modification on either the 3' or 5' end and IL-6 was sequentially injected at increasing concentrations. Only the 57-nucleotide aptamer with biotin on the 3' end displayed binding to IL-6 with this approach. Both the two-state⁶ (Figure 13 Appendix 3.1) and bivalent⁷ (Figure 12 Appendix 3.1) binding models provided equally good fits for this dataset. The data for the two-state interaction is shown in Table 1. Kinetic data could not be determined for the bivalent interaction due to its complex nature.

$k_{on1} / (M s)^{-1}$	k_{off1} / s^{-1}	k_{on2} / s^{-1}	k_{off2} / s^{-1}	K_D / M
$4.12 \cdot 10^4$	$2.32 \cdot 10^{-2}$	$2.35 \cdot 10^{-3}$	$4.60 \cdot 10^{-4}$	$92.3 \cdot 10^{-9}$

Table 1: Data for 57-nucleotide-IL-6 two-state interaction model.

SPR analysis was also conducted with IL-6 instead immobilized onto the sensor chips through amine coupling, with aptamers being sequentially injected at increasing concentrations. Both the 31- and 57-nucleotide aptamers showed good binding to IL-6, the 57-nucleotide aptamer showed a notably higher response when the biotin was on the 3' end (Figure 11 Appendix 3.1). For all these interactions, the 1:1 binding model provided the best fit. Importantly, adding a methylene blue to the 5' end of the 3' biotinylated 31-nucleotide aptamer did not prevent binding to IL-6 (Figure 14 Appendix 3.1). Conversely, adding methylene blue to the 3' of the 5' biotinylated 57-nucleotide aptamer did inhibit binding to IL-6 (data not shown). A summation of the obtained data for the best binders can be found in Table 2.

Ligand	Model	Injected analyte	$k_{on} / (M s)^{-1}$	k_{off} / s^{-1}	K_D / M	$t_{1/2} / h$
IL-6	(1:1)	3' Biotin, 5' MB 31-NT	$2.09 \cdot 10^3$	$1.04 \cdot 10^{-4}$	$4.95 \cdot 10^{-8}$	1.86
IL-6	(1:1)	5' Biotin 57-NT	$2.00 \cdot 10^3$	$1.76 \cdot 10^{-4}$	$8.81 \cdot 10^{-8}$	1.09
IL-6	(1:1)	3' Biotin 57-NT	$1.68 \cdot 10^3$	$2.16 \cdot 10^{-5}$	$1.28 \cdot 10^{-8}$	8.92
IL-6	(1:1)	3' Biotin 31-NT	$5.25 \cdot 10^4$	$5.77 \cdot 10^{-4}$	$1.10 \cdot 10^{-8}$	0.33

Table 2: Data for the best binders to immobilised IL-6 as evaluated with the 1:1 binding model ($t_{1/2}$; half-life of aptamer-IL-6 complex).

Because the 1:1 model fits these interactions it is more likely that the bivalent model holds true for the prior interaction where the aptamers were immobilised. This is because the conformational change that would be observed in the two-state model would likely be present regardless of which biomolecule is immobilised, i.e the two-state model should fit this data as well. Furthermore, it is known that IL-6 is able to form dimers in solution which could give rise to a bivalent interaction⁸.

Since only the 57-NT aptamer displayed binding when immobilised during the SPR experiments, an ELISA-inspired assay was developed to determine if the 31-NT aptamer (5' Biotin, 3' Methylene blue) could bind to IL-6 while immobilised. The aptamers were immobilised on streptavidin-coated plates, IL-6 primary, and secondary antibodies with a fluorophore were then sequentially incubated after a blocking step. A longer biotin linker on the aptamer was used here which more closely resembled, lengthwise, the linker used in the electrochemical setup. Although only qualitative, the data from this assay (Figure 5) demonstrates that the 31-NT aptamer is also capable of binding IL-6 when immobilised on a surface.

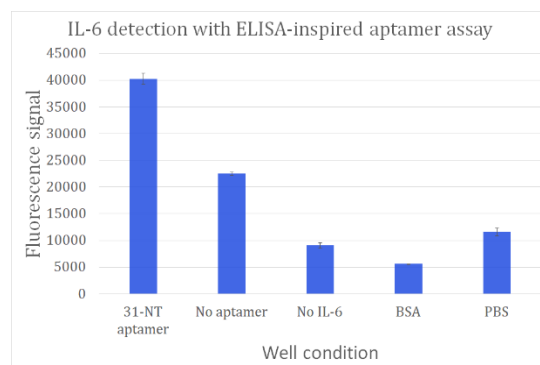


Figure 5: Fluorescence data for the differing wells from the aptamer assay. Alexa fluor 488 dye was used, λ_{ex} 488 nm, λ_{em} 523 nm.

3.2 Signal transduction

The 3' MB-31 NT-C6SH aptamer was selected based on the SPR results, that proved it as the fastest binding. Additionally, ELISA confirmed ability of the aptamer to bind IL-6 when immobilized on a surface. The electrochemical set-up is a three-electrode set-up with an aptamer-functionalized gold working electrode, an Ag/AgCl semi-reference electrode, and a silver counter electrode. In order to increase the reproducibility of the measurements, the working electrode is mechanically and electrochemically polished, to achieve a smooth surface⁹. Moreover, sonication in ethanol is performed to remove adsorbed volatile organic compounds. This is immediately followed by functionalization and blocking with 6-mercaptohexan-1-ol. The resulting functionalized electrodes are left in PBS for 12 hours to let the monolayer reorganize¹⁰.

EIS measurement is performed by setting E_{dc} at -0.27 V (equilibrium potential of methylene blue) and AC amplitude at 0.1 V and by scanning from 200000 to 1 Hz. Interaction between IL-6 and the aptamer leads to an increase in the phase shift at high frequencies (about 10000 Hz), which can be seen in Figure 6. The phase shift value after IL-6 binding is normalized as a percentage of the phase shift value before the binding. The calculated percentage of the phase shift ($\Delta\%$) is proportional to the concentration of IL-6.

The Nyquist plot (Figure 15 Appendix 3.2) depicts an increase in electron transfer resistance upon IL-6-aptamer interaction. This means that methylene blue is moved further away from the electrode surface. This behaviour is consistent with the previously observed decrease in the electric current for the same aptamer in another sensor¹¹. Further control experiments were performed to confirm that the signal is due to the IL-6-aptamer interaction. These experiments include blank measurements in the same experimental conditions, measurements with and without silicone glue and with unfunctionalized electrodes. All of them show no comparable signal. Thus, it can be concluded that the signal observed is due to IL-6 binding to the aptamer.

The resulting calibration curve displays linearity by plotting $\Delta\%$ as a function of the logarithm of IL-6 concentration (Figure 7). The dynamic range is going to be better define by further calibration.

Further improvement in terms of sample volume can be achieved by nanostructuring the working electrode. This would allow to significantly increase the surface area. Hence, it would be possible to reduce the length of the electrodes, which can be placed in a smaller cartridge. However, reproducibility and stability of such nanostructures have to be carefully assessed before implementing in a marketable biosensor.

3.3 Fluidic cartridge

The cartridge could be improved to reduce the sample volume. It would be possible by either keeping the same method and modifying the design or by completely changing the production method. For example by using physical vapour deposition on a silicon wafer or microchannels in polymers. Already different well designs are tested to see if a reduction of the sample volume could reduce the incubation time or improve the sensitivity of the sensor.

3.4 Reader instrument

Due to the low currents involved in the measurement, the potentiostat and the cartridge are placed inside an aluminium Faraday cage. This prevents interferences from external electromagnetic fields. Making the detection method more robust.

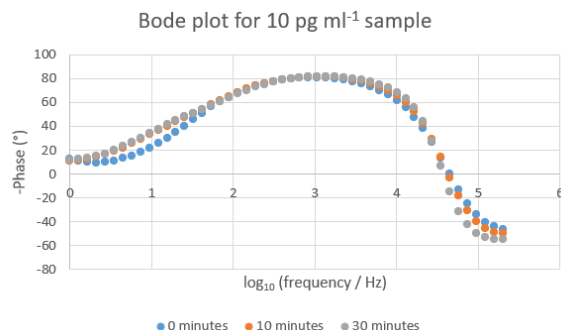


Figure 6: Bode plot recorded for 10 pg/ml in PBS at 0, 10 and 30 minutes.

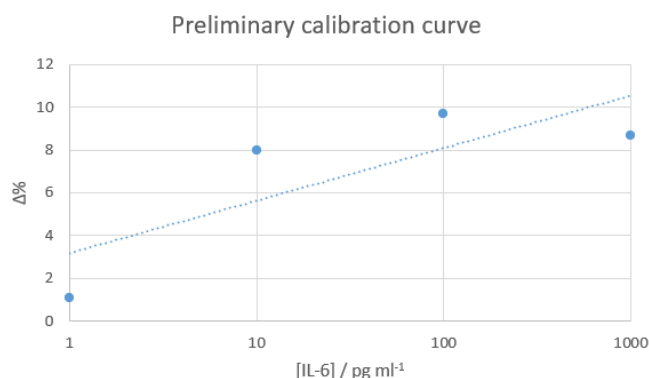


Figure 7: Preliminary calibration curve with an incubation time of 10 minutes.

4. Originality

4.1 Team Captains

Time and low production cost have been the main concerns for the UppSense team. We initially decided to develop a simple system that could be optimised in the restricted time frame and could be fast enough to analyse a sample in less than 5 minutes. We also aimed to avoid the usage of complex optical detection hardware to reduce the price of the final device. Moreover, we opted for electrochemical detection given the lower amount of interferents that can be found in plasma. With these constraints, the investigation was split into two streams focusing on antibody- and aptamer-based biorecognition, aiming to choose the most viable one.

4.1.1 Assay principle

After the literature search, one of the most feasible strategies appeared to be label-free electrochemical detection. The team decided to experiment with a state-of-art aptamer technology previously described by prof. Plaxco ⁴. After consulting Prof. Plaxco, Prof. Varghese and our supervisors, we aimed to implement an electrochemical-aptamer based (EAB) sensor for IL-6 detection.

We found several previously described aptamer sequences with affinity to IL-6 in the literature ^{2,3}. To determine best aptamer, in terms of kinetics and affinity, we used SPR and GCI in cooperation with Dr. Ewa Paul and Dr. Edward Fitzgerald, respectively. With the help of Tanay Kumar Sinha and Ehsan Manouchehri Doulabi we also adapted aptamer-based ELISA to corroborate the results ¹². The protocol was performed using the antibodies acquired from sponsors while investigating antibody-based biosensing.

When devising the final biosensor system we modified a known functionalization procedure ⁹ with additional steps to increase the reproducibility of the measurements. For detection we initially applied square wave voltammetry, the golden standard for EAB sensors. However, it resulted not suitable for our setup. Hence, we chose EIS, whose application in IL-6-EAB sensor has not been described before. It has been only used for the detection of IL-6 by addition of a $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution and by performing a washing step ³. With our device, we are able to measure directly in plasma, simplifying the procedure and the design of the device. We also devised a surface density and orientation optimisation method for the functionalized electrode.

4.1.2 Data analysis

The impedance data were displayed using the Bode plot, and the concentration was defined as a function of the change in the phase shift. The calculations were performed by a program we developed in Python.

4.1.3 Cartridge

In cooperation with Abdul Raouf Atif and Laurent Babert, we devised a low-cost, easy to assemble, original cartridge design to suit our needs. The cartridge contains three electrodes with identical lengths and fulfils the volume requirements.

4.2 Supervisors

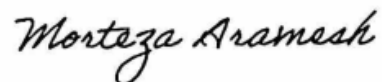
The team has received support from the supervisors and coaches during the entire project and for the development of the biosensor. We discussed a selection of the state-of-the-art methodologies and critically assessed the suitability of different methods with the team. After an extensive literature review and discussion with the expert scientists of the field, the team narrowed down the methodologies and came up with two ideas for the biosensing system and investigated the suitability of both. We provided guidance through the process of testing the two methods and evaluating their feasibility and eventually deciding on the final concept. Then the team continued on development of the read-out method by an extensive literature search as well as discussion with multiple experts, and they realized their idea with a guidance from the supervisors and coaches. The team actively collected, analysed and presented the data. The business plan was fully developed by the students with some guidance from the supervisors regarding the IP protection, and they also had several discussions with the sponsors and the experts with experience in developing successful start-up companies.

4.3 Signatures

The undersigned declares that the originality statements of the captains and supervisors are true.



Prof. Masood Kamali-Moghaddam



Asst. Prof. Morteza Aramesh



Mallika Chaukar



Ivana Suchankova

5. Translational potential

5.1 Business model canvas



5.2 Market description

Sepsis refers to life-threatening organ dysfunction caused by the dysregulated host response to infection ¹³. Sepsis has a complex mechanism, high morbidity and high mortality, posing a considerable burden on human health and socioeconomics. The development of a 'rule out' test for sepsis detection is the key to early diagnosis and the premise for improving the cure rate of patients affected by sepsis.

According to statistics, the global sepsis diagnostics market is valued at USD 719 million in 2022. It is expected to continue to grow at a compound annual growth rate (CAGR) of 9.2% during 2022-2028, reaching USD 1226 million by 2028 ¹⁴. While the European market size reached USD 430 million this year, with a compound annual growth rate of 8.43%, and it is expected to reach 645 million USD in 2027 ¹⁵. To enter this market, UppSense biosensor needs to follow EMA approval rules. The medical device market in EU countries is regulated by Regulation (EU) 2017/745 and In-Vitro Diagnostic Devices Regulation (IVDR). Our biosensor is described as a Class I medical device according to the regulations. At the same time, to commercialize a product suitable for the medical device market, CE certification must also be obtained.

5.3 Stakeholder desirability

Sepsis is a global emergency that causes over 20% of global deaths, killing 11 million people yearly. In 2020 World Health Organization (WHO) declared sepsis a global concern with an urgent need for better treatment and diagnosis ¹⁶.

Although the early diagnosis of sepsis has been crucial for sepsis management, current diagnostic routine is acutely slow and is mainly based on non-specific symptoms ¹⁷. Biomarkers of acute inflammation, such as C-reactive protein, procalcitonin, lactate and IL-6 have currently been used for sepsis diagnosis ¹⁸.

From these biomarkers, IL-6 was found to be the most specific for the detection of an early-stage sepsis ^{19,20}. Despite its potential usefulness, the standard IL-6 ELISA can take up to 24 hours. Our point-of-care IL-6 biosensor would reduce the handling and assay time to maximum of 20 minutes.

The biosensor enables rapid quantitative detection of IL-6 in blood plasma, which is a reliable and inexpensive solution to the current shortcomings in sepsis prediction. At the same time, its small size and portability make it possible to equip the sensor in different locations such as hospital wards and ambulances. Biosensors are simple to operate, save training time costs, and enable rapid detection for patients (see Figure 16 Appendix 5.3, our value proposition), enabling early diagnosis and treatment of sepsis.

To illustrate the difference this device would make on sepsis management we described a patient journey (see Figure 8, our patient journey). The aim is to reduce the detection time of sepsis, enable point-of-care monitoring, and thereby give early treatment and increase recovery rates. Since the market is lucrative for biosensing to detect early stage sepsis for millions of patients worldwide, the competition will be large. According to the market analysis from Mordor Intelligence ²¹, there are already two companies, namely Beckman Coulter Inc. (Danaher Corporation) and T2 Biosystems, Inc., which reached clearance with the FDA for their diagnostics tools. None of these companies is using IL-6 as a biomarker. Along with using the IL-6, most specific biomarker for sepsis, our device will also be adaptable and multiplexable. Using the same detection method, we can detect several different biomarkers, such as IL-10 and procalcitonin. The detection of multiple biomarkers can further improve the accuracy of sepsis diagnosis. We believe our product can not only fill a gap in today's market, but also contribute to reducing sepsis mortality.

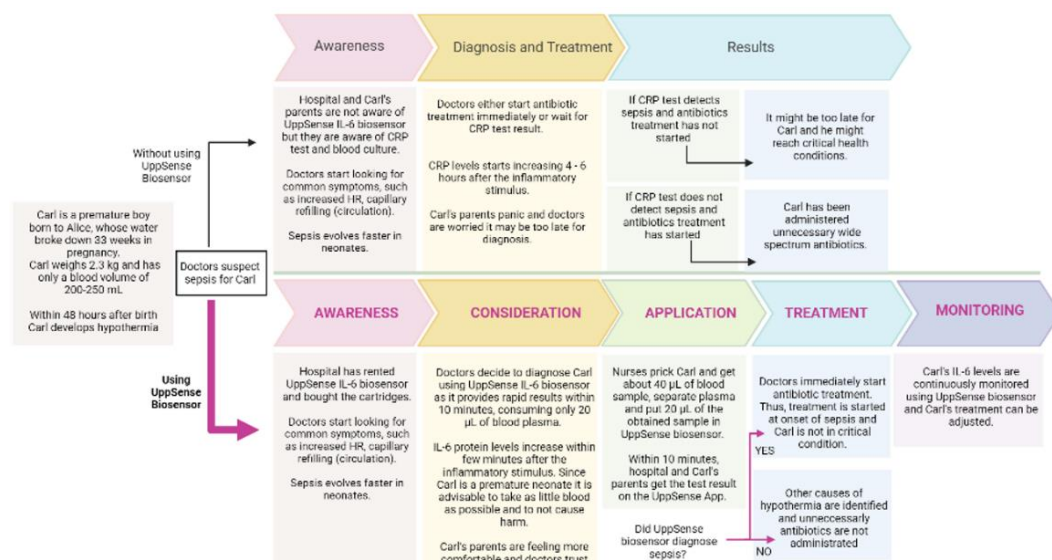


Figure 8: Patient journey for the UppSense IL-6 biosensor (HR: heart rate).

5.4 Business feasibility

Our team will leverage the fruitful start-up environment at Uppsala University (UU) and Uppsala Innovation Center (UIC), which was recently ranked by UBI Global as the World Top 5 Public Business Incubator. As the first step, our team will devise a proof-of-concept of the EAB-IL-6 assay, focusing on its sensitivity and reproducibility of the measurements. This will be financed through research grants, provided by UIC or Swedish Research Council. Once the proof-of-concept is successfully described, we will launch a start-up and immediately apply for broad patent protection with the help of the UU Innovation Hub. The UppSense start-up will then create a flexible team including the CEO, a regulatory manager, engineers and scientists, a marketing strategist, and CFO (see the SWOT analysis in Appendix 5.2).

The prototype and the business model will be developed hand-in-hand with UIC by the end of 2024. Both will be based on in-depth conversations with diagnostics expert prof. Anders Larsson, hospital management and clinicians working at the ICU units of the Uppsala academic hospital (one of the partners of the UIC), and Karolinska Hospital, Stockholm. A strategic partnership could also be formed between Uppsense and Uppsala-based companies such as Gradientech and Q-Linea, which have recently successfully launched diagnostic devices on the market. Depending on the final design of the prototype, disposable cartridges may require additional patent protection as they are thought to be the main source of profit according to our current business model.

As the next step, we will prepare for the first investment application. This requires a prototype, sufficient patent protection, and statement of key opinion leaders. We are planning to apply for financing from our local Swedish investors such as governmentally oriented Almi invest (Allan Asp) or

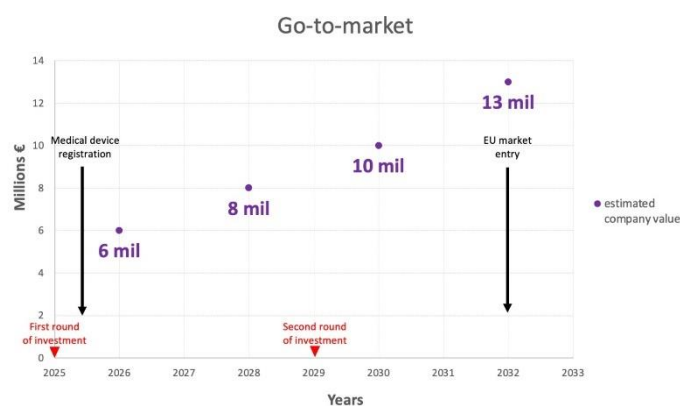


Figure 9: Projected company value growth estimates starting from the first round of capital investment in 2025. The initial company value is based on a discussion with prof. Jons Hilborn. The following values were calculated based on the the average PE-ratio of the industry and our projected revenue based on the Table 3.

a private investor Drivhuset. Approximately one year will be needed to build the strong foundation of the investor relationship. With the first investment and all the required documents, our first-generation medical device will obtain Class I Medical device registration in MA (in approximately 6 months), and the CE marking, which will allow our biosensor to enter the whole European market. We will then perform pilot studies with key opinion leaders in Sweden, and in the next stage in France and Germany. The return of the first investment is expected in 4-5 years (Figure 9).

Second stage investment round will provide resources for building a cartridge manufacturing plant and production scale-up. The manufacturing process will rely on some of our current key partners. IDT showed interest in becoming an exclusive supplier of modified oligonucleotides for large-scale production. Sustainability goals are met through the fast chemical synthesis of aptamers with no need of cooling during storage²². Uppsala Therapeutics offered to synthesize oligonucleotides with special modifications. Tailor-made potentiostat can be manufactured through the partnership with Palmsens. The electrochemical sensor hardware and software will be developed in cooperation with Zimmer & Peacock. We are now establishing distributor relationships with Malvern Panalytical, with the help of Dr. Edward FitzGerald.

5.5 Financial viability

The financial viability of UppSense is supported by a business strategy built on surveys and interviews with academics, prof. Jöns Hilborn, prof. Anders Larsson, Prof. Ulf Landegren, physicians, Magnus Engberg MD., Fredrik Ahlsson MD. and investors from Drivhuset, and UIC. The cartridges we design make up our revenue stream. The estimated material cost of sensors and cartridges is €560/unit and €0.92/unit respectively, and can be reduced by mass production. An additional manufacturing cost has also been accounted for. The sensor device will be leased with nominal charge, while the cartridges will be sold for €25/unit. In Sweden, there are 1,178 hospitals and primary health care centres. Assuming that each hospital and primary health care centre are interested in leasing at least two biosensors, that

would entail a saturated market at 2,356 biosensors. This is most likely a low estimate, since larger hospitals would require more biosensors for their operations. In 2021, approximately 25,000 IL-6 tests were requested by such institutions. These tests were mainly administered for sepsis and COVID-19 diagnosis. However, tests of this nature are not routinely administered for such diagnoses. This leaves room for expansion in IL-6-based tests. The financial assessment (see Figure 10 and Table 3 Appendix 5.4) therefore expects a total of 2,550 leased biosensors and 50,000 cartridges per year after 10 years of operations in Sweden. The financial assessment also accounts for a € 100 per year cost of installation and maintenance of the leased biosensors. This is also expected to cover the depreciation of the biosensors, which have an anticipated life expectancy of 5-7 years.

During the first years of operation, a substantial part of operating expenses will be research and development (R&D). This is to further optimize the biosensor, cartridges, and manufacturing process. As revenue and profit margins increase, increases in spending on R&D will ensure enough capital to continuously improve the biosensor, and expand the product catalogue.

After the two first years of the development phase, revenue streams will be established. High revenue growth will be established in the first years with a steady decrease in revenue growth rate as the company grows. In 2032, ten years of operations, expected revenue will amount to € 1,390,000 with a net profit of approximately € 330,000. With expansion into the broader European market (Table 4 Appendix 5.4), with an estimated market size surpassing € 630 million, future growth is secured¹⁵.

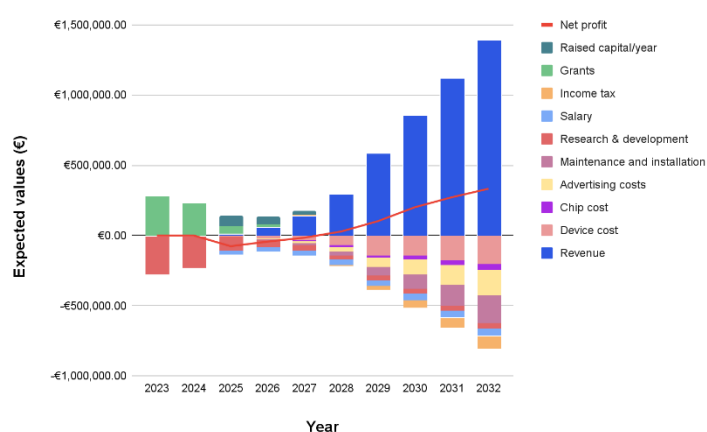


Figure 10: Financial outlook for the initial ten years of operation. Estimates for revenue, net profit, and cost structure expressed in euros.

6. Team and supports

6.1 Team

Evangelia was part of the sub-team focused on finding aptamers for the detection of IL-6. Especially, she tried to optimise ELISA using aptamers. Also, she contributed to kinetic studies and functionalization of electrodes.

Paula was in charge of the Social media and IT team. She took care of the website, LinkedIn, and Instagram accounts, while the second was the implementation of the software for the biosensor.

Mallika was co-captain, involved in the management and organisation of the team. She communicated with the key figures and contributed in the development of immunoassays, affinity studies and electrochemical detection.

Jasmine was part of the sub-team focused on finding aptamers for the detection of IL-6 and contributed to the kinetic studies via aptamers ELISA. Additionally, she was in charge of ordering all the supplies for the project.

Chiara was part of the IT team. She was involved in the development of the software. Furthermore, she was contributing to team organisation, deadlines and logistics. She also contributed in the business case.

Jesper contributed in designing and engineering of the cartridges. He was also involved in the development of a business model and in contacting companies and potential investors.

Kristian was part of the sub-team designated to investigate immunoassay techniques. He also contributed with electrodes development, affinity studies, commercialization potential and contact with partners and organisers.

Joel was mostly involved in the characterisation of the aptamer-IL-6 interactions with SPR and GCI. He also performed ELISA-inspired assays with the aptamers and contributed by contacting people within academia.

Favour was involved in the sub-team researching aptamers. She also contributed on the electrochemical detection method. She also was in charge for contacting companies and placing orders for the team T-shirts.

Giorgio designed and developed the functionalized electrodes and optimised the detection method. He also provided scientific advice for software development, cartridge design, affinity studies and business case.

Ivana was co-captain, involved in the strategy development and organisation of the team. She communicated with key figures and provided scientific support on bioinformatics, affinity studies and oligonucleotide design.

Jacome was involved in the sub-team focusing on IL-6 detection with the use of antibodies. He developed the strategy, detailed the protocols, performed the assays and approached companies for sponsorships.

Luc has been in charge of the designing and assembling of the chip and general sensor around the chosen method of detection. Deciding the methods and materials to use. He was also taking part in the business case.

Qingyu was responsible for producing and processing pictures and videos and assisted in the preparation of the electrodes. At the same time, he was also the main player in charge of the commercialization plan.

Paolo was part of the sub team focused on affinity and kinetic characterization. He helped devise ELISA-related tests and worked in close contact with experts, coordinating the experiments with both Cytiva and Creoptix.

6.2 People who have given support¹

Our supervisors Prof. **Masood Kamali-Moghaddam** and Asst. Prof. **Morteza Aramesh** both gave us scientific advice and essential feedback on our work and provided lab facilities and contacts on researchers in the area. Guidance on electrochemistry were provided by Asst. Prof. **Alina Sekretareva**. Prof. **Kevin Plaxco** provided useful information about the technology of EAB sensors. **Abdul Raouf Atif** and Dr. **Laurent Barbe** guided us in the development of the cartridge as well by providing access to instrumentations. Dr. **Ewa Pol** provided access to SPR instrumentation and guidance in the design of the related experiments, Dr. **Edward Fitzgerald** had the same role for the experiments with GCI. **Tanay Kumar Sinha**, **Ehsan Manouchehri Doulabi**, Prof. **Ulf Landegren** and Prof. **Anders Larsson** supervised the team in the development of the immunoassay experiments. Dr. **Annete Roos** guided us in the choice of the characterization techniques and provided contacts to experts in the field. Prof. **Jöns Hillborn**, Dr. **Sara Petterson** and Dr. **Olivia Tolman** provided guidance regarding intellectual property, business development and related bureaucratic processes. Prof. **Oommen Varghese** provide advice on the design of the aptamers, stability and affinity studies. **Magnus Engborn MD** and **Fredrik Ahlsson MD** explained the sepsis diagnosis procedure used in hospitals and provided related statistics.

6.3 Sponsors

IDT provided high quality functionalized aptamers. Hytest provided IL-6 samples. Palmsense provided electrochemical instrumentation. ProteinTech, Sinobiological, GeneTex, Atagenix provided antibodies. Cytiva and Creoptix provided guidance and instrumentation for the affinity studies. Future Diagnostics provided guidance on immunoassays.

¹ Affiliations provided in appendix 6.1

7. Final remarks

The UppSense team was able to develop an EAB sensor for IL-6 detection. The aptamer was selected after literature screening and characterization with multiple techniques. The detection was performed via EIS, which was never applied before in IL6-EAB sensor detection. This resulted in a linear response over three orders of magnitude, with an incubation time of 10 minutes. Furthermore, the team developed a business model for the commercialization of the biosensor.

In the future, we are planning some more experiments regarding electrochemistry, affinity studies, and bioinformatics to publish a research paper.

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9. Appendix

3.1 SPR sensorgrams



Figure 13: IL-6 (93.75-1500 nM) binding to the immobilized 57-nucleotide (3' Biotin) aptamer fitted to the two-state model.

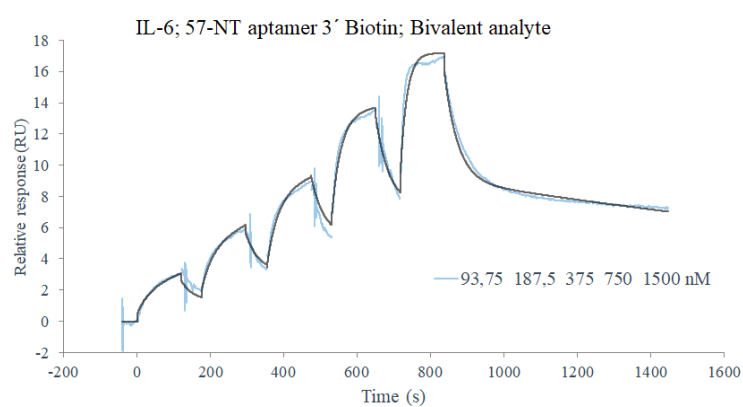


Figure 12: IL-6 (93.75-1500 nM) binding to the immobilized 57-nucleotide (3' Biotin) aptamer fitted to the bivalent model.

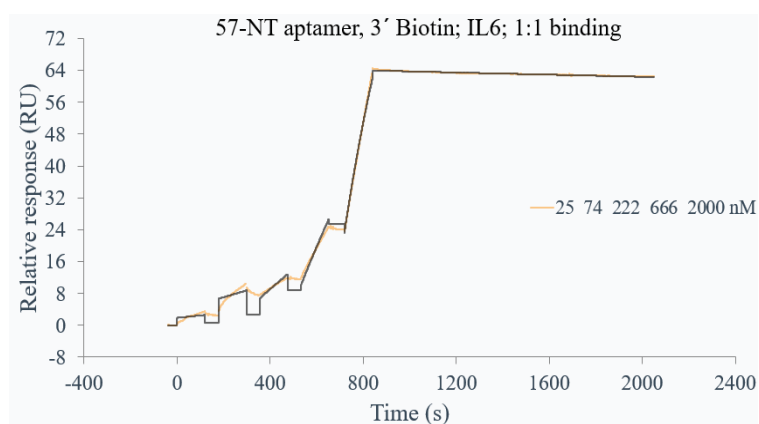


Figure 11: 57-nucleotide aptamer (3' Biotin) (25-2000 nM) binding to immobilized IL-6.

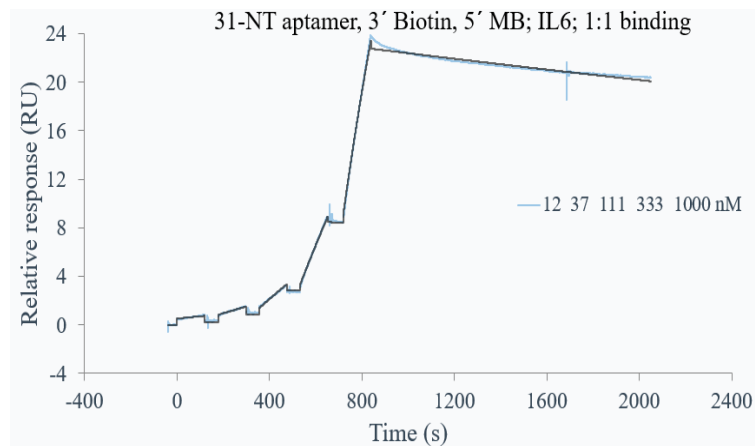


Figure 14: 31-nucleotide aptamer (3' Biotin, 5' MB) (12-1000 nM) binding to immobilized IL-6.3

3.2 Electrochemical data

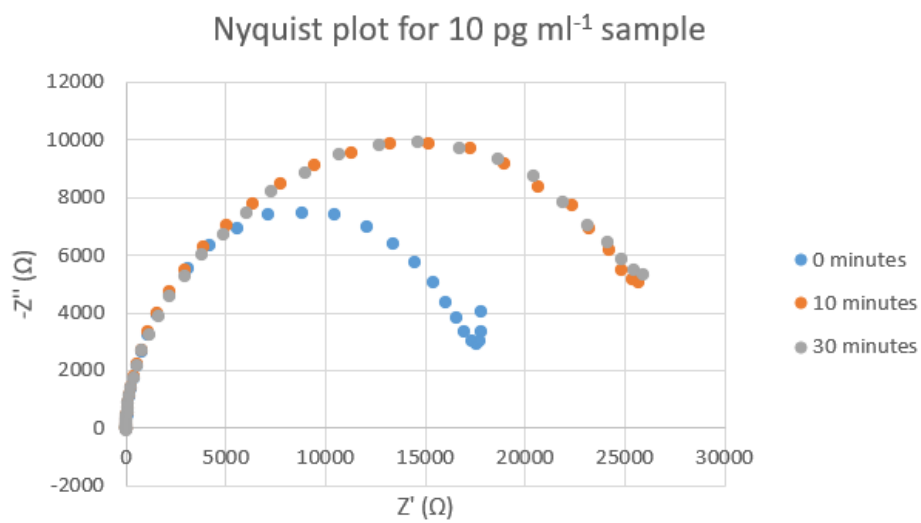


Figure 15: Nyquist plot for a 10 pg ml⁻¹ sample of IL-6 measure in PBS at 0, 10 and 30 minutes.

5.1 Customer journey

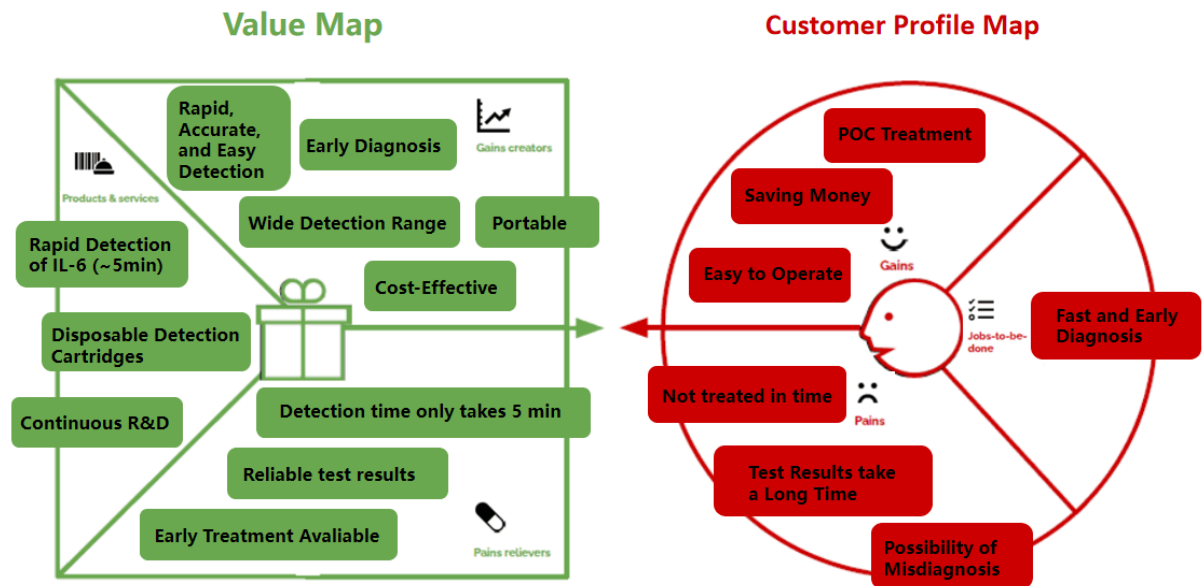
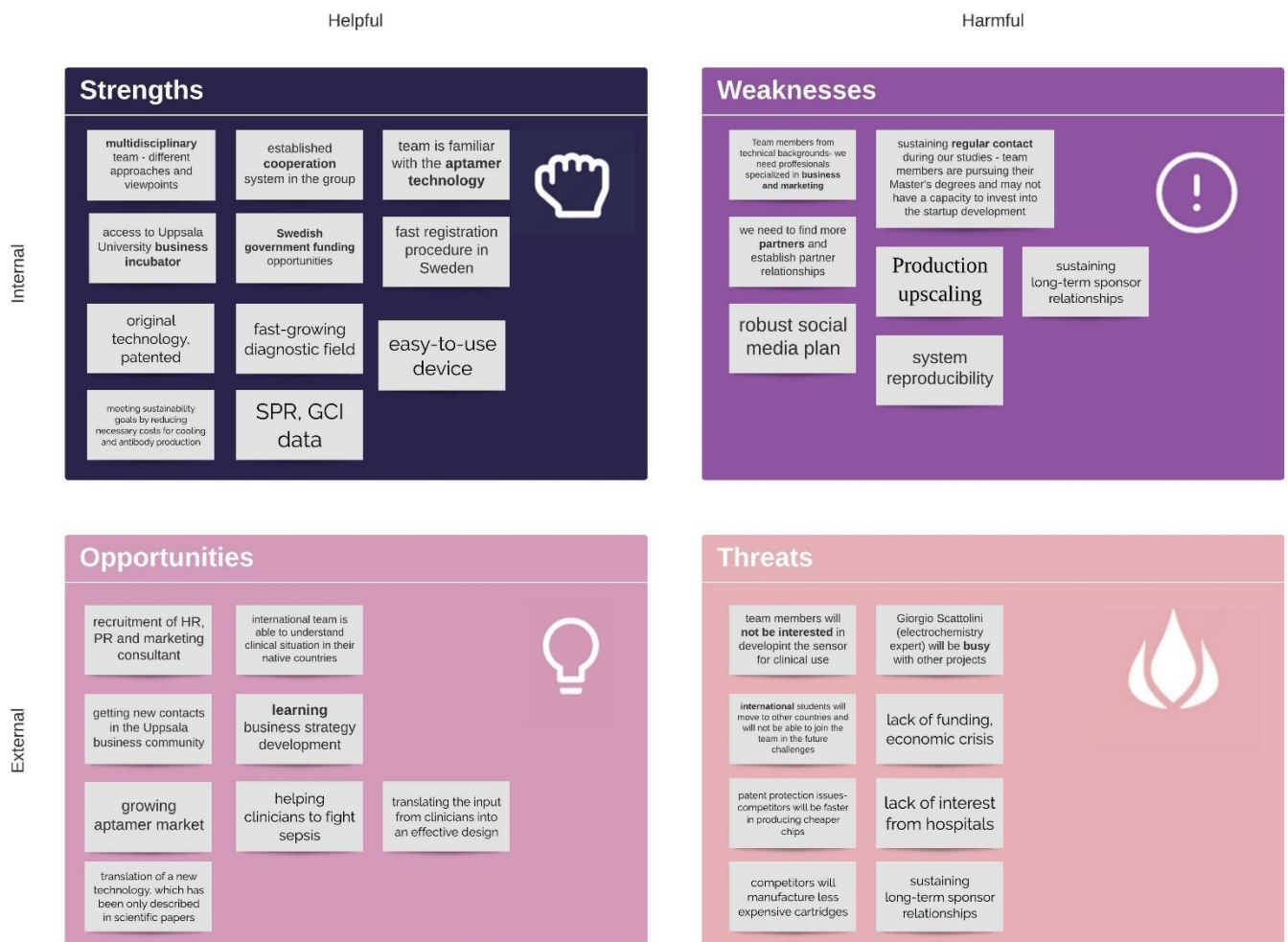


Figure 16: Value proposition for the proposed business model.

5.2 SWOT analysis



5.4 Financial viability data

	Development phase		Sweden expansion phase							
	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032
Revenue	0	0	14,500	58,000	145,000	290,000	580,000	850,000	1,120,000	1,390,000
Total cost of revenue	0	0	8,878	34,088	59,520	123,040	249,600	306,400	396,800	494,600
- # of devices on lease	0	0	10	50	150	350	750	1,250	1,850	2,550
- Device cost	0	0	5,600	22,400	35,000	70,000	140,000	145,000	174,000	203,000
- # of cartridges sold	0	0	500	2,000	5,000	10,000	20,000	30,000	40,000	50,000
- Cartridge cost	0	0	460	1,840	4,600	9,200	18,000	27,000	34,000	42,500
- Production cost	0	0	1,818	4,848	7,920	15,840	31,600	34,400	41,600	49,100
- Maintenance & installation	0	0	1,000	5,000	12,000	28,000	60,000	100,000	147,200	200,000
- Salaries	0	0	30,000	33,000	36,300	39,930	43,923	48,315	53,147	58,462
Gross profit	0	0	5,622	23,912	85,480	166,960	330,400	543,600	723,200	895,400
Operating expenses	275,000	231,000	131,124	87,782	93,396	103,322	141,503	190,110	233,508	276,120
- Salaries	0	0	30,000	33,000	36,300	39,930	43,923	48,315	53,147	58,462
- Research & development	275,000	231,000	100,000	50,000	40,000	30,000	31,500	33,075	35,721	38,579
- Advertising	0	0	1,124	4,782	17,096	33,392	66,080	108,720	144,640	179,080
Grants	275,000	231,000	50,000	25,000	0	0	0	0	0	0
Raised capital/year	0	0	80,000	50,000	25,000	0	0	0	0	0
Pretax income	0	0	-76,502	-43,870	-19,916	35,638	128,897	253,490	342,492	419,280
Income tax	0	0	0	0	-4,103	7,341	26,553	52,219	70,553	86,372
Net profit	0	0	-76,502	-43,870	-15,813	28,297	102,344	201,271	271,939	332,908

Table 3: Financial expectations for the initial ten years of operations. Estimates for revenue, net profit, and cost structure.

Selected countries in the EU	Population	Revenue (€)	Net profit (€)
Sweden	10,350,000	1,390,000	330,000
Germany	80,520,000	3,513,920	834,240,
France	65,630,000	6,171,600	1,465,200
Italy	63,730,000	6,950,000	1,650,000
Spain	59,680,000	1,441,486	342,223
Poland	38,530,000	692,359	164,373

Table 4: Expected revenues and net profits from selected EU countries.

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