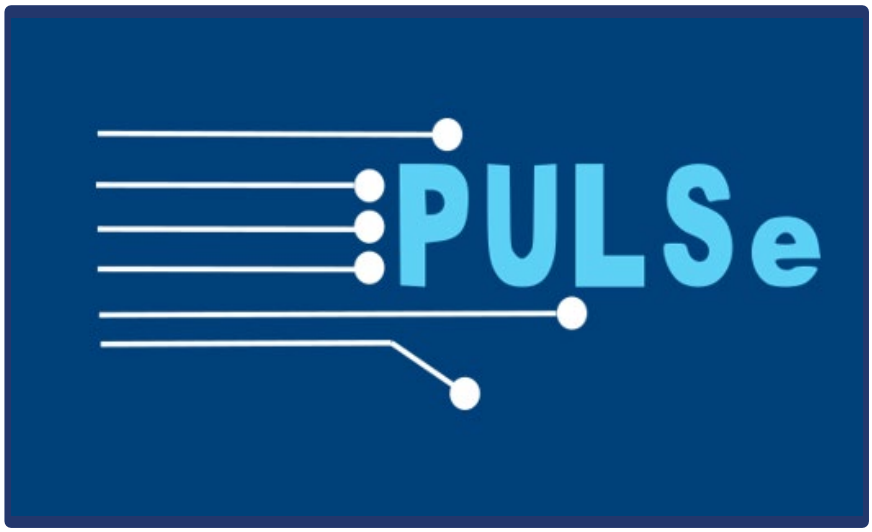
Team Results Document



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12 August 2022

KU LEUVEN

SensUs

Contents

1. Abstract

The Point of Care University of Leuven SensUs team (PULSe) consists of an international group of students studying in a variety of technology, biology, and engineering related fields. Over the past several months we have used our diverse backgrounds and skill sets to work towards a common goal: developing a biosensor for sepsis detection using interleukin-6 (IL-6) as the biomarker.

Here, we present a fast (<10 min) and easy to use sensor that can be used in hospitals or urgent care centers to provide early diagnosis and screening of sepsis in high-risk patients. To use our system, a 10 µL plasma sample is loaded into the microfluidic cartridge and activated with a single finger press. The plasma mixes with antibody functionalized gold nanoparticles (AuNP) already present in the cartridge and is guided by the microfluidic channels towards a fiber optic (FO) sensing element. The optical fiber is prepared prior to insertion in the cartridge with a thin gold layer (~50 nm, Au) and functionalized with capture antibodies. Once the mixed sample interacts with this fiber, all elements bind, resulting in a sandwich of capture antibody, IL-6 and detection antibody bound to AuNP. The binding of these elements to the fiber is detected using surface plasmon resonance (SPR), measuring the wavelength shift over time to obtain a kinetic measurement.

2. Biosensor System and Assay

An overview of the bioassay, transduction method, and readout of our SPR-based biosensor is shown in Figure 1. Each of these individual components will be described in more detail in the following sections.

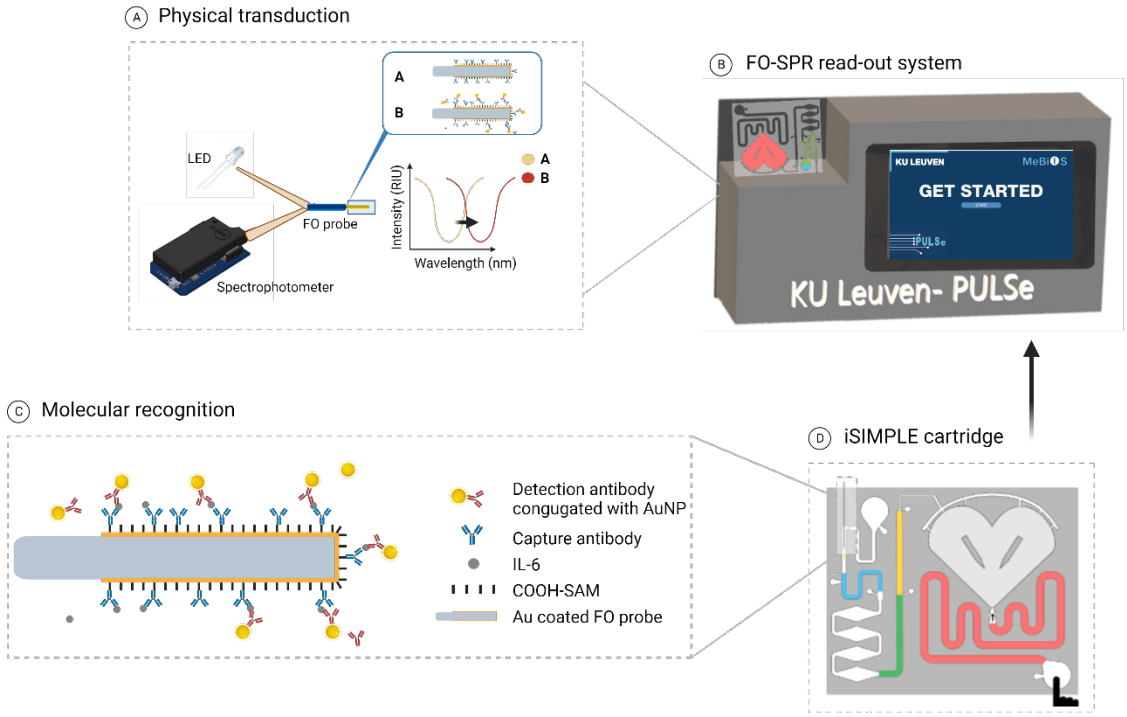


Figure 1. Schematic overview of the IL-6 biosensor (IL-SENSE) developed by PULSe. (A) Physical transduction mechanism, (B) the FO-SPR read-out system, (C) the molecular recognition system, and (D) the iSIMPLE cartridge (Sepcards).

2.1 Molecular Recognition and Assay Reagents

To accomplish sensitive detection of IL-6 in plasma, a sandwich immunoassay was designed. FO probes were coated with a 50 nm Au layer and functionalized with a carboxylic acid self-assembling monolayer (COOH-SAM), followed by the immobilization of Anti-IL-6 capture antibodies to the probes using EDC-NHS chemistry. While these fibers can be used for direct IL-6 detection without further assay steps, higher sensitivity was required to get into the clinically relevant range. To amplify the signal, it was decided to implement a sandwich assay using AuNPs functionalized with detection antibodies. Both capture and detection antibodies were monoclonal (provided by HyTest, Finland), to prevent cross-reactivity and increase the specificity. A schematic for the molecular recognition system is shown in Figure 1C.

2.2 Physical Transduction

To translate the IL-6 binding into a measurable signal, FO-SPR was chosen as the transduction mechanism. SPR is a popular technique to measure the association and dissociation rate in analyte-ligand interactions in real time as well as for the rapid quantification of analyte concentrations (Piliarik, Vaisocherová, & Homola, 2009).

In SPR, light causes the excitation of electron density oscillations (known as surface plasmon wave, SPW) at a metal-dielectric interface. In our biosensor, this is formed between the Au coating of the FO probe and the surrounding medium including the components of the immunoassay. When sending light through the FO probe, resonance occurs when the energy and momentum of the incident light matches that of the SPW. This results in a sharp dip in the reflected light intensity that can be measured with a spectrometer (Gupta, Banshi & Verma,

2009). The binding of an analyte in solution to its receptor immobilized on the sensor surface results in a local change of the refractive index (RI) of the surrounding medium, which in turn causes a shift in the resonant wavelength. As opposed to traditional prism based SPR systems, SPR sensors using FO probes allow a compact and low-cost, miniaturized system with remote sensing capability (Zeni, L, et al; 2020).

2.3 Cartridge Technology

To improve user-friendliness, the functionalized FO probe 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 established by the MeBioS-Biosensors research group at KU Leuven (Dal Dosso et al., 2018). The main characteristic of this approach is the on-chip pumping mechanism, which consists of a porous material (filter paper) that uses capillary imbibing to move the working liquid. This creates a positive pressure in the channels, that can be used to push liquids through the chip.

Our chip is comprised of a working liquid chamber, mixing region, and analytic chamber. The mixing region is similar to the design of Qu et al. (2022) and is used to combine the sample with functionalized AuNPs on the chip. The mixed sample is then pushed to the analyte chamber is where it interacts with the FO probe. Prior to reading the sample, the FO probe is washed with PBS, which was pre-loaded onto the chip. Each of the regions and working liquids are shown along with the chip's layer-based composition in Figure 2.

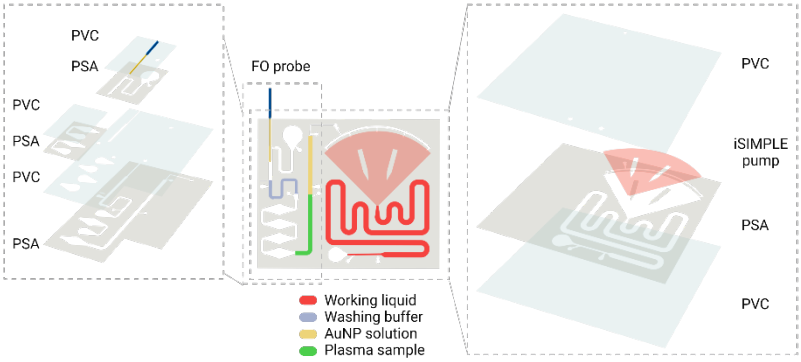


Figure 2. Schematic overview of the iSIMPLE chip with the three chambers: working liquid chamber, mixing region and analytic chamber and their composition of multiple layers of PVC and PSA.

2.4 Instrument Readout and User Interaction

The FO-SPR setup of our sensor contains a LED (LUXEON C White 3000K, Lumileds, USA) that shines light onto an optic fiber that guides it to the sensor tip. The reflected light is captured by a UV/VIS Micro Spectrometer (Insion, Germany). Both the spectrometer and LED light are connected to and commanded by a Raspberry pi 3 model B. The measurements are shown on a 7-inch Raspberry Touch display. All this is contained in a 22 cm x 22 cm x 8 cm box made of robust plastic material.

The iSIMPLE chip is connected to the port at the top of the device and a plasma sample is placed at the sample inlet. The user then activates the chip, and the IL-6 measurement is then started from the touch screen. As the sample approaches the probe and fills the detection site, the program measures the intensity of the reflected light every half second and finds the resonance dip with its corresponding wavelength. The slope of the resonance wavelengths over time is then compared to a calibration curve contained in the program and an IL-6 concentration value is displayed on the screen. Finally, the chip is removed and properly disposed. The entire procedure takes less than 10 minutes per sample.

3. Technological feasibility

3.1 Molecular Recognition and Physical Transduction

The feasibility of the bioassay was tested using the WhiteFox 1.0 (FOx Biosystems, Belgium) FO-SPR device. First, the assay performance was evaluated by measuring different IL-6 concentrations in PBS, then samples in human plasma were used to assess the effect of potential unspecific binding reactions. A hyperbolic equation was used to fit a dose-response curve describing the shift in FO-SPR wavelength in function of IL-6 concentration.

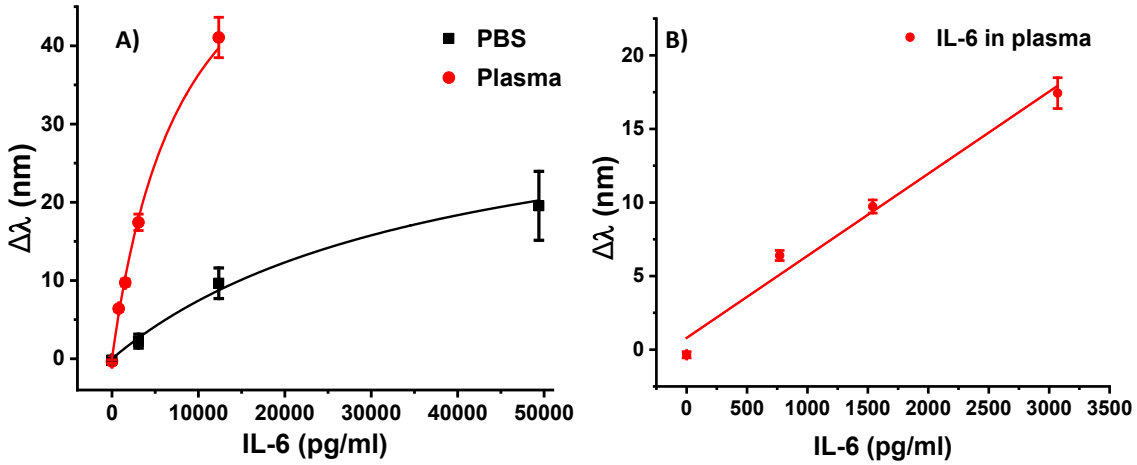


Figure 3. A) Dose-response curve (FO-SPR wavelength shift versus IL-6 concentration). A hyperbolic calibration curve was fitted to measurements in PBS (R^2=0.9729) at IL-6 concentrations 0, 3080, 12350, and 49390 pg/ml and in human plasma (R^2=0.9895) was measured at concentrations 0, 770, 1540, 3080, and 12350 pg/ml. Error bars represent standard deviation (n=3). **B)** Dose-response curve (FO-SPR wavelength shift versus IL-6 concentration), dynamic range (0-3080 pg/ml). A linear equation was used to perform calibration (R^2=0.9664).

The preliminary endpoint measurements were performed in PBS and in plasma, respectively. The curves showed an initial linear phase followed by saturation at high IL-6 concentration. Human plasma did not show significant unspecific binding (α=0.05), moreover, the sensitivity of the assay improved compared to PBS. This underlines the specificity of our assay and shows potential for application of the assay in a point-of-care IL-6 biosensor. To reduce the time to result in the final device, kinetic data analysis will be performed.

To estimate the limit of detection (LOD=3.3*SD/slope), linear fitting was applied at the clinically relevant concentration range (0-3080 pg/ml) which overlaps with the dynamic range of the assay. The LOD was found to be 133.7 pg/ml which allows the assay to be suitable for sepsis diagnosis and detection of systemic inflammation stage 2.

We aim to further improve the sensitivity of our assay and estimate the LOD on the final chip construct. The chip design and architecture will significantly reduce the required sample volume and minimize the user interaction, ensuring a shorter measurement time.

3.2 Cartridge Technology and Instrument Readout

As previously mentioned, an iSIMPLE chip was chosen as the platform to manipulate the sample and reagents for carrying out the concentration measurements. The design is based off the work presented by Qu et al. and is shown in Figure 4 (Qu et al. 2022). As a proof of concept of our instrument readout system, tests were performed using different sucrose concentrations (0, 3 and 6wt%). The principle of the set-up of the instrument readout system was the same as how it will be used in the competition, except that the fiber was not functionalized with capture antibodies.

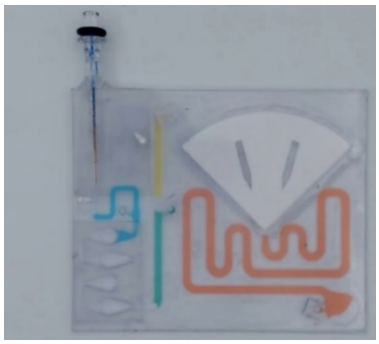


Figure 4. Sepcard with preloaded liquids

During the measurement process, the software records the reflected intensity at each wavelength between 320-1200 nm at half second intervals until the measurement is stopped. An example of the intensity vs wavelength over time is shown in Figure 5A. A clear dip in intensity is visible in this figure, which is the resonant wavelength. In the case where there is analyte binding to the surface, the resonant wavelength will shift to higher values over time as more analyte is bound. For the sucrose measurements, there is no binding to the surface, so a relatively constant value is expected. These results are shown in Figure 5B for a 3 wt% sucrose solution.

The results of the sucrose measurements show that the chip produces the expected trends for the conditions tested and that the methods of producing the chip and inserting the fiber do not impede its functionality.

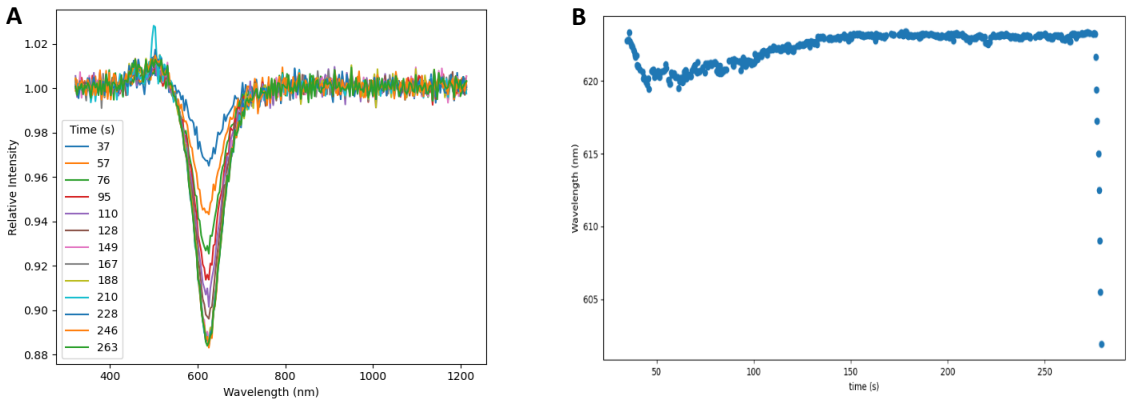


Figure 5. Measurement of a 3 wt% sucrose solution using the IL-SENSE and Sepcard. **A)** shows the intensity for the wavelengths scanned (shown at time intervals of 20 second for visibility). **B)** shows the resonant wavelength over time.

4. Originality

Team: In literature, most biosensing concepts for IL-6 detection are based on electrochemistry, using antibodies and aptamers as recognition elements coupled with nanostructures for signal amplification (Chen, 2017; Khan, 2020; Tertis, 2017). With regards to optical biosensors, SPR has been scarcely explored for IL-6 determination and FOs have been utilized (Battaglia et al., 2005) developed an FO-SPR biosensor for IL-6 detection in saline solution and in in cell culture media. However, to the best of our knowledge, there have not been any reports of SPR biosensors for IL-6 detection that are incorporated with a microfluidic platform. For this competition, we developed a sandwich bioassay type FO-SPR based biosensor with antibodies used for IL-6 recognition coupled with excellent optical properties of AuNPs and integrated into a self-powered microfluidic chip. Connecting the FO-SPR to the iSIMPLE chip opens the possibility for IL-6 detection outside the conventional laboratory setting as such an approach merges the analytical advantages of FO-SPR and flexibility of the portable iSIMPLE system. Furthermore, we have assembled a portable readout system consisting of commercially available optical components which are connected to form an integrated, ready-to-use instrument. The system emphasizes user friendliness with ease of handling being a priority during development. This is most evident in the iSIMPLE chip which incorporates a sensing probe connected to the instrument and contains all the necessary reagents for IL-6 determination. Thus, the user must only add the sample solution and initiate the measurement by a single press on the iSIMPLE cartridge. The acquisition and processing is automated with the results being displayed on the screen without the need for further data handling. Therefore, the determination of IL-6 concentration can be performed in a timely manner and without the need for special training. Consequently, the process of sepsis diagnosis by IL-6 determination is moved away from analytical laboratories to medical facilities where swift and accurate POC biosensing is a valuable resource and boosters the quality of medical care.

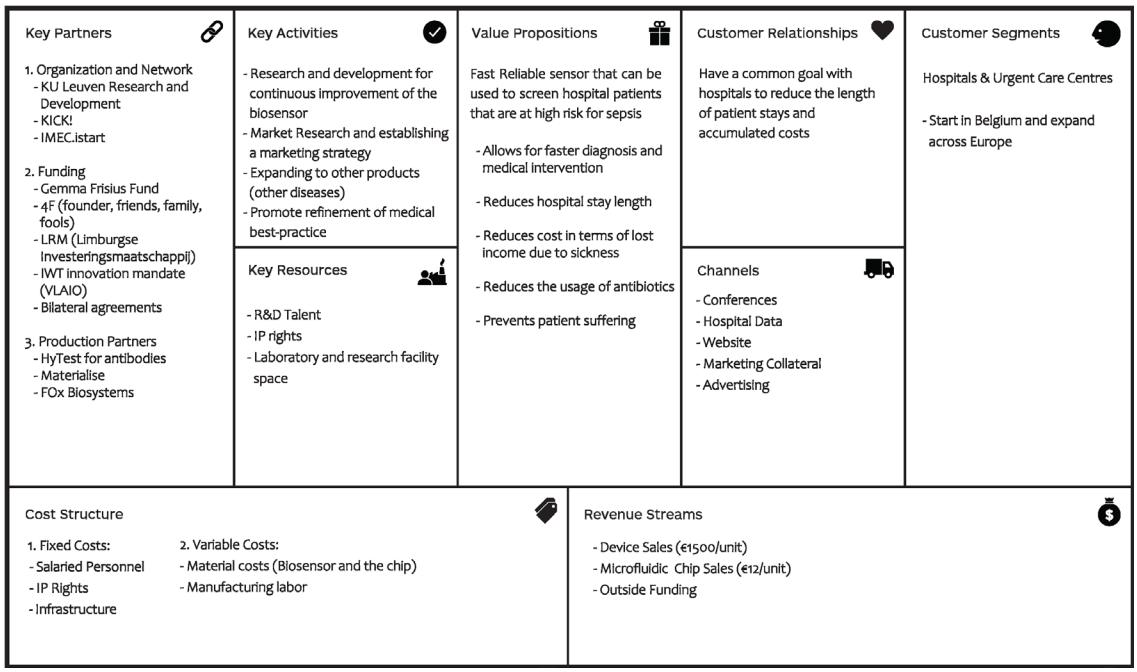
Supervisor: Throughout the process of developing the proposed biosensing concept, the team received support from us, the supervising professor and a team of four coaches. More specifically, in the initial phase of the project, we discussed a selection of technologies and biosensing concepts with the team, inspired by the expertise and materials available within our group. Next, based on these concepts, an extensive literature search and their own interests and knowledge, the team came up with several ideas for the biosensor. Subsequently, we guided them in making the final selection of the biosensing and readout principle. The initial plan was an aptamer-based sensing concept due to its novel nature, however this concept had no success due to a lack in signal upon target binding. Despite this major setback, halfway through the competition the team was able to persevere and 'plan B' was executed: an antibody sandwich assay inspired by previous work done in the lab by Ordutowski *et al*. (2022). Finally, the team ended up with a feasible biosensing concept that was slightly less novel, however able to detect IL-6 in a sensitive manner. The supervisors provided protocol drafts and training in the lab, in order to facilitate the autonomy of the students throughout the competition. Afterwards, the students planned, performed, and interpreted the experimental results independently. A weekly team meeting with the coaches was organized to discuss the progress and plan subsequent experiments to further optimize the biosensor and to help the students in making the right choices. The business plan related to this biosensing concept was fully developed by the students by looking for information independently and organizing discussions with sponsors and experts in the biosensing field. During this process, the students had access to TRDs from previous KU Leuven teams to get an idea of what the business plan could look like. Several iterations of the business plan were discussed with the coaches to further improve the work.

Supervisor: Jeroen Lammertyn

Team Captains: Mislav Matic & Jessica Zeman

5. Translation potential

5.1 Business Model Canvas



5.2 Market Description

Sepsis is commonly defined as a life-threatening medical condition that originates from a dysregulated immune response to infection. While severe complications disproportionately affect the very young, elderly, and immunocompromised individuals, sepsis has a large global impact (Gotts and Matthay, 2016). According to a study referencing data from the Global Burden of Diseases, Injuries and Risk Factors Study (GBD), there were an estimated 48 million cases of sepsis and 11 million sepsis related deaths worldwide in 2017 (Rudd et al., 2020).

Looking at the effects and resources associated with severe sepsis, it has both a large humanistic and economic toll. Hospital stays related to sepsis are among the longest (typically 75% longer than stays related to other conditions), and most expensive, as acute conditions require more hospital resources further increasing the cost (Paoli, 2018). Patients who survive sepsis can also have long-term physical and psychological problems which add to the toll on their quality of life (Tiru et al., 2015).

One of the best ways to reduce the negative side-effects of sepsis, is by diagnosing and beginning treatment early before it progresses to widespread organ disfunction or failure. According to the Surviving Sepsis Campaign, an international organization focusing on developing best practices for reducing mortality and morbidity associated with sepsis, the first item on their recommendations from 2021 were to provide “sepsis screening for acutely ill, high-risk patients […]” (Evans et al., 2021). Currently, blood analysis and cultures used to diagnose sepsis are rather extensive. Therefore, the patient needs to a have a confirmed or suspected infection as well as two of the following criteria: elevated respiratory rate, low systolic blood pressure or a score less than 15 on the Glasgow coma Scale before starting testing (Evans, 2018).

Consequently, proactive screening is not feasible with the current analysis methods and leaves an opening in the market for biosensors. With our IL-6 biosensor, it would be possible to effectively screen high-risk patients during their hospital stay in an affordable manner.

5.3 Stakeholder Desirability

Looking at the market and scope of the problem, there are several stakeholders who would take an interest in finding new solutions to reduce the cost of sepsis, including hospital staff such as doctors and nurses, patients, hospital administration, and insurance companies.

From the perspective of the healthcare provider, the main goal is to minimize patient stays and detect disease early to allow for intervention. By using a fast and easy to use biosensor, patients that have an increased risk for sepsis complications, can be tested on a routine basis as a preventative measure rather than waiting until they have signs of sepsis. This can reduce costs associated with unnecessary testing and decrease the length of the hospital stay and resources required for sepsis patients. This is also beneficial from the hospital management perspective and of course for the patients themselves. As insurance companies partially or totally cover the hospital cost, they would also have an economic incentive to improve preventative diagnostics and early intervention for diseases. With shorter hospital stays, and less acute care required, the hospital bills would be lower for the insurance companies. This saves money in terms of both the cost of the hospital stay, and lost work/productivity due to prolonged illness. From the patient perspective, this not only decreases the risk of death, but also helps reduce sick time and lasting side-affects due to illness.

To sum up, compared to current methods, implementing a biosensor as a preventative screening method to detect sepsis would help alleviate pain for the patient and increase gains for all stakeholders, from hospital staff to insurance companies.

5.4 Business Feasibility

PULSe is a team of three technical experts in sensorics, microfluidics and bioassay development. Our start-up is supported by scientific advisors of MeBioS KU Leuven, specialized in FO-SPR technology. Besides that, our main expertise relies on the in-house developed SIMPLE chip technology. Moreover, our customer service will help to integrate our system into the electronic charting system of the healthcare provider. The hardware production (device) will be manufactured by our sponsor, FOx-Biosystems, while reagents (e.g., antibodies) will be purchased from pharmaceutical companies (e.g., HyTest). The commercialization takes place in four phases.

Phase I. – Incubation phase (2022-2024)

As the team initially will be in a university environment, we will be supported by the expertise and network of KICK and the KU Leuven's R&D department to manage the start-up in the incubation phase. During the first years, we will in close collaboration with our key opinion leaders, clinical specialists of Department of Intensive Care Medicine, UZ Leuven and ULB-Erasme to clinically validate our system. Their feedback will ensure to customized and user-friendly platform. To enhance engagement with the development phase, our product will be offered free of charge. Two other hospitals (UZ Gent, UCL Saint-Luc) will be targeted with a reduced price, at a market share of 1.5%. Further hospitals will be reached out at professional events and conferences to build a network within Belgium. The incubation phase ends with the foundation of PULSe, leaving the university environment, and entering the market with our product.

Phase II. - Launch in Belgium (2025-2026)

As customer (and patient) satisfaction is of high importance, we aim to fine-tune and further develop our product based on their feedback. This will be done by database building. The initiation of marketing activity will enhance reaching out to 3rd party collaborators. The market share is planned to be increased from 1% (beginning of phase) to 3.5% in Belgium by the end of this phase.

Phase III. - Business expansion (2027-2028)

In the first year of third phase, we aim to enter foreign markets, initially focusing on the Netherlands and Luxemburg to establish our company within the Benelux. New customers will be reached out at professional events and increased marketing activity. The market share in the new countries will initially be 1.5%, then in one year it will be increased to 3.5%. In the second year of this phase, we will aim to enter the broad European market as well. During this phase, the profit of the company is re-invested to develop new products (markers for different diseases). These new products will help us to increase the yearly income, as they will be all compatible with the device.

5.5 Financial Viability

The financial viability of PULSe will depend on both our now developed sensor and later sensors based on our concept. We have spoken to Laurent Vancaillie, Venture Development manager at IMEC and Francesco Dal Dosso, innovation manager at KU Leuven to have a better idea on the financial aspect of a start-up company. Our three main initial revenue streams will come from the device itself (the IL-SENSE), the cartridges (Sepcards) needed for the device and from two rounds of funding, €3 million seed funding at the start of our incubation phase in 2022 and another €1,1 million when we launch our product in 2025. Since the focus in the first two-three years will lie on further developing and optimizing our concept into a working product, our biggest expense will be in the R&D. The founder of PULSe will employ a postdoc R&D engineer and one PhD student to limit the headcount cost. Since we are initially linked to KU Leuven, we can make use of its facilities during the first two years. After that, our facility will be placed at an incubator, BioVille Limburg.

In the launching phase (2025), the IL-SENSE will cost €1100 while the cartridges will be sold for €6/piece, both close to the manufacturing price (respectively €1068 and €3,6). In this way, the first hospitals will be encouraged to use the product and perform more tests before the actual treatment. This, on the longer run, can impact the current medical protocols and lead to more sustainable healthcare. To conquer the Belgian market in 2026, where the selling price for the IL-SENSE will increase to €1500 and the cartridges will cost €12/piece. The OpEx is kept relatively low in this way for the hospitals, while their CapEx will be charged to the patients. During the expansion phase in 2027, we will have additional expenses to cover marketing and sales activity.

From 2028 onwards, the European market will be our focus area, with 3,4 million patients in total. We expect expenses for sales personnel, traveling, marketing and legal administration. At the same time the number of products sold in the EU will double compared to 2027 and it will keep increase linearly afterwards. The break-even point will be reached in 2029, 4 years after the launch of our first product. The detailed cost calculations can be found in appendix 9.4 together with a schematic overview in Figure 6.

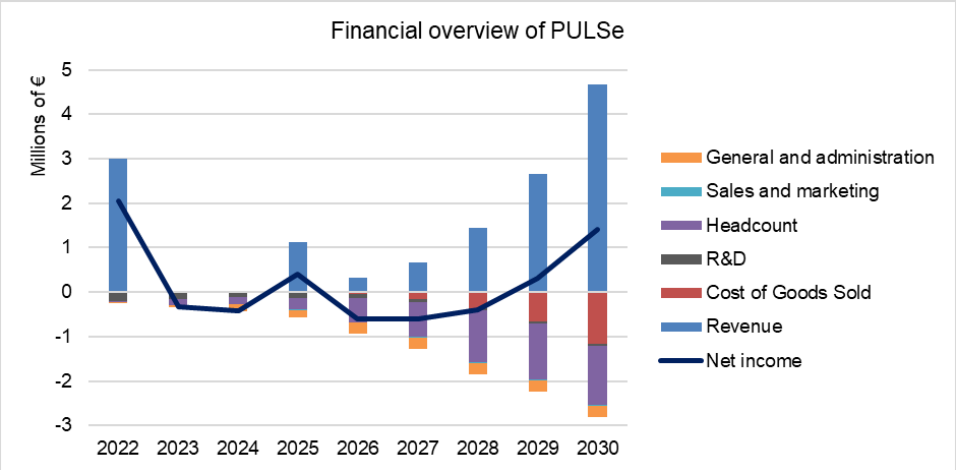


Figure 6. Overview of the estimated revenue, cost projection and net income for the first years of operation.

6. Team and support

6.1 Contributions of the Team Members

Amine Eda Yeşilyurt: Member of bioassay and communication teams
Aniruddh Holemadlu: Member of technology team
Anjana Balachandran: Member of bioassay team
Aurora Pierucci: Member of bioassay and communication teams
Aída Fernández: Member of technology and sponsoring teams
Cyrille Sébert: Member of technology and sponsoring teams
Héloïse Ameel: Member of bioassay team and member of the business team
Jessica Zeman: Captain of the bioassay team and member of the business team
Mathias Huybrechts: Member of technology and sponsoring teams
Mislav Matic: Captain of the technology team and member of the communication teams
Tamas Trombitas: Member of bioassay, sponsoring and business teams
Tutku Çalık: Member of bioassay and sponsoring teams

6.2 Additional Support

Professor Jeroen Lammertyn is the head of the MeBioS-biosensors group at KU Leuven. He arranged for our participation in the SensUs competition and allowed us to use his laboratory space, materials, and equipment for the development of our biosensor. Without all of this, our participation in the SensUs competition would not be possible.

All our coaches were PhD candidates within Professor Lammertyn's group. They provided critical knowledge, support, and advice for helping us realize our biosensor design. **Seppe Driesen** and **Aurélie Mohrbacher** were specifically involved in coaching the bioassay sub-team, while **Wannes Verbist**, and **Simão dos Santos** focused on the technology team.

We would also like to thank **Yagmur Yildizhan** for her advice on working with antibodies as a detection element and **Annelies Dillen** for sharing her experience in working with FO sensing elements and the FOx machines. Additionally, we would like to thank **Ruben Cops** for the safety rules and regulation training for working in the laboratory.

Finally, we would like to thank **Kathleen Zeman**, a nurse, for her insights into the medical treatment of sepsis patients, **Laurent Vancaillie**, Venture Development manager at IMEC and **Francesco Dal Dosso**, innovation manager at KU Leuven for their insights about the business plan.

6.3 Sponsors

FOx Biosystems sponsored PULSe financially, and their technology was used to validate the bioassay component while our transduction element was still under development.

The KU Leuven **Luc Sels Fund** provided funding for our stay and travel to Eindhoven for the competition.

7. Final Remarks

Our aim for this competition was to develop a reliable and accurate biosensor for IL-6 detection. In doing so, we utilized a known bioassay concept to bolster good analytical performance and combined it with a microfluidic platform to create our unique device. In our design, we focused on user friendliness and cost-effectiveness and tried to make the determination of IL-6 concentrations as simple as possible by optimizing the readout system.

We envisioned the device to be used in hospitals and urgent care centers, and outside the conventional laboratory setting to increase the quality of healthcare available to those in need. In the future, we would like to focus on additional trials to test the true potential of our biosensor and to optimize it further to make it a viable product for routine point-of-care use.

We would like to thank the SensUs program for organizing the competition, and KU Leuven for giving us the opportunity to participate in it. It has helped us get a deeper understanding of both the technical and business challenges associated with developing a new biosensor. We would also like to thank Professor Lammertyn for his advice and our coaches from the MeBioS group at KU Leuven for their guidance. They were of immense help in the development process. Furthermore, we extend our gratitude to our business partner, FOx Biosystems, for giving us insights into our biosensor development and financial resources.

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9. Appendix

9.1 Make-up of the Microfluidic Chip

The microfluidic chip consists of different polyvinylchloride layers (PVC) joined together with pressure sensitive adhesive (PSA). Whatman filter paper grade 598 was used for the porous pump and hydrophobic air vents. The microfluidic channels and inlay for the porous pump were cut out the PSA layer with a laser cutter.

9.2 Use of the Microfluidic Chip

Operation of the cartridge can be described by the four main steps shown in Figure A1. First, the different liquids of the system are pre-loaded via four specific inlets. These liquids and their respective inlets are indicated on Figure A1-A. After loading the liquids and blocking the inlets, the chip can be activated by means of a single finger press until the working liquid reaches the pump (Figure A1-B). The third step consists of the automated mixing of the sample and the functionalized AuNP solution as well as washing of the probe with PBS (Figure A1-C). Finally, the sample reaches the probe, and the readout step can occur (Figure A1-D).

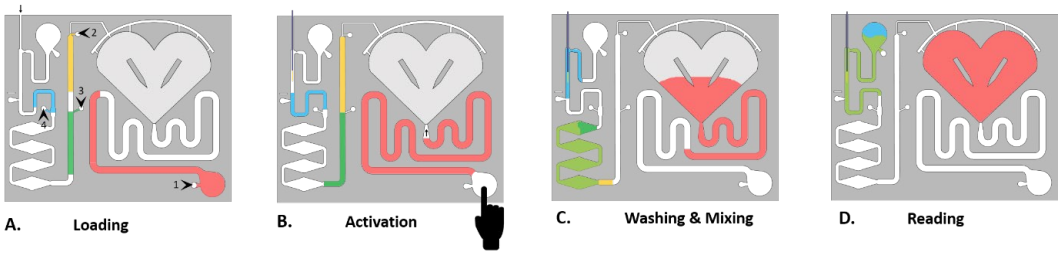

Figure A1. An overview of the steps in operating the microfluidic cartridge **A)** Liquids are loaded into the device. Each of the inlets for these liquids are labeled as: 1. working liquid (red), 2. Secondary antibody-AuNP complex solution (yellow), 3. Plasma sample (green), and 4. Washing PBS (blue). **B)** Flow of the liquid is activated by pressing the cartridge, **C)** Sample is mixed with the antibody coated AuNPs and FO probe is washed with PBS, **D)** Device can begin recording the spectrum intensity once the sample reaches the FO probe

9.3 Overview of financial viability

	2022	2023	2024	2025	2026	2027	2028	2029	2030
Revenue	*3.000.000*	*1*	*1*	*1.125.837*	*322.962*	*667.786*	*1.453.115*	*2.651.255*	*4.677.822*
Cost of Goods Sold	0	-1427,58	-1787,16	-16301,94176	-54920,7962	-167167,6934	-356877,1692	-652631,7046	-1170793,61
R&D	-200000	-150000	-120000	-120000	-90000	-50000	-50000	-50000	-50000
Headcount	-15333,33333	-142140	-146404,2	-260069,026	-540869,735	-784373,0675	-1158317,73	-1266486,787	-1306498,61
Sales and marketing	-5000	-5000	-5000	-7000	-9000	-15000	-25000	-25000	-25000
General and administration	-32500	-32500	-152500	-171000	-241000	-251000	-251000	-251000	-251000
Net income	***2.060.375***	***-331.068***	***-425.691***	***413.600***	***-612.828***	***-599.754***	***-388.080***	***304.603***	***1.405.897***

9.4 Detailed cost calculation of financial viability

PULSe																	
				Incubation phase				Launch in Belgium		Belgium		Benelux		Launch in Europe		Europe	
		2022 (sep-dec)		2023		2024		2025		2026		2027		2028		2029		2030	
Revenue	in EUR																		
Initial product																			
Sepstop - Cartridge	Unit Price	0,00		0,00		0,00		6,00		12,00		12,00		12,00		12,00		12,00	
	#Units	0,00	0,00	0,00	0,00	0,00	0,00	3939,58	23637,48	13788,53	165462,36	35413,21	424958,52	70826,42	849917,04	120404,91	1444858,97	215312,32	2583747,80
Sepstop - Machine	Unit Price	0,00		0,00		0,00		1100,00		1500,00		1500,00		1500,00		1500,00		1500,00	
	#Units	0,00	0,00	0,00	0,00	0,00	0,00	2,00	2200,00	5,00	7500,00	24,00	36000,00	48,00	72000,00	96,00	144000,00	192,00	288000,00
Expansion product range																			
New disease	Unit Price											15,00		15,00		15,00		15,00	
	#Units		0,00		0,00		0,00		0,00		0,00	13788,53	206827,95	35413,21	531198,15	70826,42	1062396,30	120404,91	1806073,71
Funding																			
Bilateral agreements			1500000,00																
IWT innovation mandate (VLAIO)			1000000,00																
Gemma Frisius Fund			500000,00																
4F (founder, friends, family, fools)									50000,00										
imec.istart									50000,00		150000,00								
LRM									1000000,00										
Net sales			3000000,00		0,00		0,00		1125837,48		322962,36		667786,47		1453115,19		2651255,27		4677821,51
Costs of goods sold																			
Outsourcing of production																			
Cartridge	Unit Price	3,60		3,60		3,60		3,60		3,60		2,88		2,88		2,88		2,88	
	#Units		0,00	100,00	359,58	200,00	719,16	3939,58	14165,94	13788,53	49580,80	35413,21	101871,06	70826,42	203742,11	120404,91	346361,59	215312,32	619376,02
Machine	Unit Price	1068,00		1068,00		1068,00		1068,00		1068,00		1068,00		1068,00		1068,00		1068,00	
	#Units		0,00	1,00	1068,00	1,00	1068,00	2,00	2136,00	5,00	5340,00	24,00	25632,00	48,00	51264,00	96,00	102528,00	192,00	205056,00
Cartridge - 2	Unit Price											2,88		2,88		2,88		2,88	
	#Units											13788,53	39664,64	35413,21	101871,06	70826,42	203742,11	120404,91	346361,59
Total cost of goods sold			0,00		1427,58		1787,16		16301,94		54920,80		167167,69		356877,17		652631,70		1170793,61
GROSS PROFIT (LOSS)			3000000,00		-1427,58		-1787,16		1109535,54		268041,56		500618,78		1096238,02		1998623,56		3507027,90
Operating expenses																			
Headcount																			
CEO	Cost per year	100000,00		103000,00		106090,00		109272,70		112550,88		115927,41		119405,23		122987,39		126677,01	
	#FTE	0,00	0,00	0,00	0,00	0,00	0,00	1,00	109272,70	1,00	112550,88	1,00	115927,41	1,00	119405,23	1,00	122987,39	1,00	126677,01
CTO	Cost per year	90000,00		92700,00		95481,00		98345,43		101295,79		104334,67		107464,71		110688,65		114009,31	
	#FTE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	101295,79	1,00	104334,67	1,00	107464,71	1,00	110688,65	1,00	114009,31
Finance & HR manager	Cost per year	90000,00		92700,00		95481,00		98345,43		101295,79		104334,67		107464,71		110688,65		114009,31	
	#FTE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	107464,71	1,00	110688,65	1,00	114009,31
Experienced R&D engineer (Post-doc)	Cost per year	90000,00		92700,00		95481,00		98345,43		101295,79		104334,67		107464,71		110688,65		114009,31	
	#FTE	0,33	30000,00	1,00	92700,00	1,00	95481,00	1,00	98345,43	1,00	101295,79	1,00	104334,67	2,00	214929,41	2,00	221377,30	2,00	228018,61
R&D engineer (Phd student)	Cost per year	48000,00		49440,00		50923,20		52450,90		54024,42		55645,16		57314,51		59033,95		60804,96	
	#FTE	0,33	16000,00	1,00	49440,00	1,00	50923,20	1,00	52450,90	3,00	162073,27	3,00	166935,47	3,00	171943,53	3,00	177101,84	3,00	182414,89
Lab technician	Cost per year	50000,00		51500,00		53045,00		54636,35		56275,44		57963,70		59702,61		61493,69		63338,50	
	#FTE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Sales	Cost per year	0,00		0,00		60000,00		61800,00		63654,00		65563,62		67530,53		69556,44		71643,14	
	#FTE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	63654,00	3,00	196690,86	5,00	337652,64	5,00	347782,22	5,00	358215,69
Marketing	Cost per year	0,00		0,00		0,00		60000,00		63000,00		66150,00		69457,50		72930,38		76576,89	
	#FTE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	66150,00	1,00	69457,50	2,00	145860,75	2,00	153153,79
Admin	Cost per year	0,00		30000,00		30000,00		30000,00		30000,00		30000,00		30000,00		30000,00		30000,00	
	#FTE	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	30000,00	1,00	30000,00	1,00	30000,00	1,00	30000,00
			46000,00		142140,00		146404,20		260069,03		540869,74		784373,07		1158317,73		1266486,79		1306498,61
Research and development																			
R&D costs			200000,00		150000,00		120000,00		120000,00		90000,00		50000,00		50000,00		50000,00		50000,00
			200000,00		150000,00		120000,00		120000,00		90000,00		50000,00		50000,00		50000,00		50000,00
Sales and marketing																			
Marketing and sales cost			0,00		0,00		0,00		2000,00		4000,00		10000,00		20000,00		20000,00		20000,00
Website			5000,00		5000,00		5000,00		5000,00		5000,00		5000,00		5000,00		5000,00		5000,00
			5000,00		5000,00		5000,00		7000,00		9000,00		15000,00		25000,00		25000,00		25000,00
General and administrative costs																			
Infrastructure			0,00		0,00		0,00		36000,00		36000,00		36000,00		36000,00		36000,00		36000,00
IP			22500,00		22500,00		22500,00		45000,00		45000,00		45000,00		45000,00		45000,00		45000,00
IT consultant			0,00		0,00		60000,00		10000,00		10000,00		10000,00		10000,00		10000,00		10000,00
Advisory council			0,00		0,00		0,00		0,00		0,00		0,00		0,00		0,00		0,00
Travel, food, phone			0,00		0,00		0,00		10000,00		20000,00		30000,00		30000,00		30000,00		30000,00
Legal advisor			0,00		0,00		60000,00		60000,00		120000,00		120000,00		120000,00		120000,00		120000,00
Undefined			10000,00		10000,00		10000,00		10000,00		10000,00		10000,00		10000,00		10000,00		10000,00
			32500,00		32500,00		152500,00		171000,00		241000,00		251000,00		251000,00		251000,00		251000,00
TOTAL OPERATING EXPENSES			283500,00		329640,00		423904,20		558069,03		880869,74		1100373,07		1484317,73		1592486,79		1632498,61
EARNING BEFORE TAXES			2716500,00		-331067,58		-425691,36		551466,51		-612828,17		-599754,29		-388079,71		406136,78		1874529,29
Cumulated loss (profit)	in EUR		2716500,00		-331067,58		-425691,36		551466,51		-612828,17		-599754,29		-388079,71		406136,78		1874529,29
Taxable basis	in EUR		2716500,00		0,00		0,00		551466,51		0,00		0,00		0,00		406136,78		1874529,29
Corporate taks	25%		679125,00		0,00		0,00		137866,63		0,00		0,00		0,00		101534,19		468632,32
NET INCOME			2037375,00		-331067,58		-425691,36		413599,88		-612828,17		-599754,29		-388079,71		304602,58		1405896,97
Cumulative profit/dept			2037375,00		1706307,42		1280616,06		1694215,94		1081387,77		481633,48		93553,77		398156,35		1804053,32

9.5 User Interface