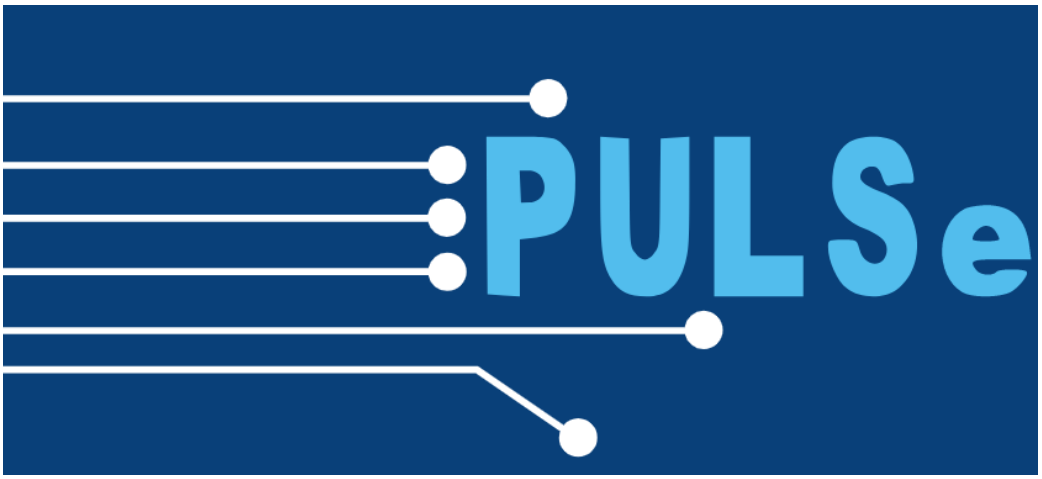
Team Results Document

PULSe



Team members:
Darina Abaffyová
Eléonore Wolters
Anaïs Hubart
Evelyn Lerinckx
Emily Rosschaert
Ivana Janíčková
Jintong Ge
Lai-Jin Lily Leung
Maxim Lambrechts
Vasilis Loukopoulos
Wout Mens

Supervisor:
Prof. Jeroen Lammertyn

Coaches:
Aida Monserrat Pagès
Charlotte van Tricht
Lorenz Van Hileghem
Wannes Verbist
Yagmur Yıldızhan

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KU LEUVEN

SensUs

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Summary

PULSe is the Belgian team of students representing KU Leuven. Driven by their passion for science, the team has developed the PULSe platform, a user-friendly biosensor for detecting hemagglutinin, a protein of the H1N1 influenza virus, in saliva.

The platform, consisting of a disposable cartridge and a docking device, can be used after downloading an application and connecting the smartphone to the docking device. The user needs to deposit saliva into an ergonomic funnel connected to the cartridge. The app will display the instruction to slide the cartridge into the docking device. In this way the cartridge slides over a system of electromagnets, moving magnetic beads that are functionalized with MNAzyme from the first to the second reservoir through a channel. As a result, the beads firstly react with saliva in the first reservoir. Then, in the second reservoir, beads cleave the beacons and produce a fluorescent signal. Finally, the smartphone detects the signal and automatically displays the quantitative result after data processing.

This document aims to show the feasibility of the concept based on literature research, preliminary data and modeling. We believe our concept can be materialized and become a must-have tool for controlling the spread of flu.

Biosensor System and Assay

Molecular Recognition and Assay Reagents

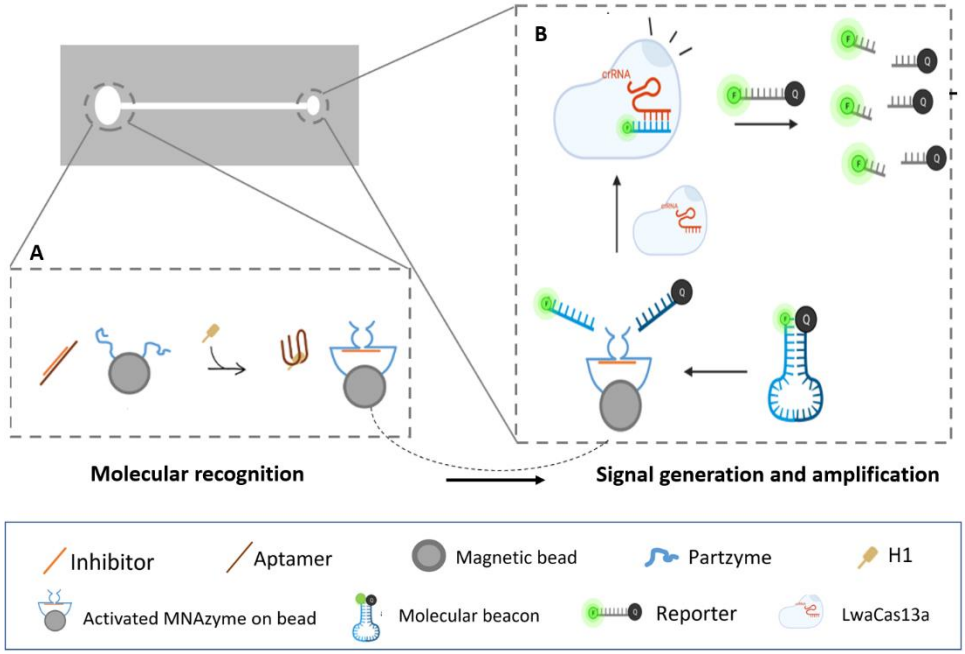


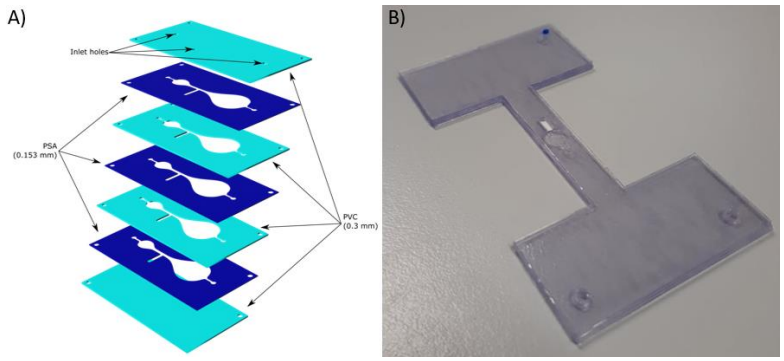
Figure 1. *Schematic diagram of salivary H1-protein detection based on aptamer recognition, MNAzyme and CRISPR-Cas13 signal amplification.* ***A)*** *Binding of H1 to the aptamer triggers the dehybridization of the aptamer and its corresponding inhibitor. Subsequent activation of the MNAzyme by hybridization with the released inhibitor.* ***B)*** *Molecular beacon cleavage by the activated MNAzyme. Fluorescent tagged beacon arm activating the CRISPR-Cas13 complex, which in turn cleaves the reporter molecule generating the amplified signal.*

The driving force of the biosensor is the innovative combination of three concepts: aptamers, multi-component nucleic acid enzymes (MNAzymes) and the CRISPR-Cas13 technology (Figure 1). Aptamers are nucleic acids (NAs) which can show high affinity and specificity towards their ligands (Song *et al.*, 2012). They have considerable benefits, such as low cost, thermal stability, and design flexibility. The aptamer specific to hemagglutinin (H1) forms a complex with an inhibitory strand. When the target is present, the aptamer binds to it, causing dehybridization of the inhibitor sequence.

MNAzymes are catalytically active NA complexes consisting of two partzymes (Figure 1A). Each partzyme has a facilitator binding arm, a substrate binding arm, and a catalytic core. The MNAzyme facilitator-binding arms are designed in such a way that they are complementary to the inhibitor. This inhibitor thus functions as the facilitator strand that binds the facilitator binding arms of the partzymes to form a catalytically active MNAzyme complex (Figure 1A). MNAzymes break the bond between two RNA bases, resulting in the need for a full single-stranded RNA (ssRNA) substrate. Cleavage only occurs when the MNAzyme complex is assembled, and the substrate can anneal to the substrate binding arms. In this assay, the RNA substrate that binds the MNAzyme is a molecular beacon that contains a stem and a loop structure. The stem contains the fluorophore-quencher pair (Figure 1B). Due to the cleavage of the molecular beacon, the stem region dehybridizes, thereby separating the fluorophore and the quencher, which results in an increase in fluorescent signal. An important effect of the cleavage is the resulting ssRNA in the solution, which is integral to further signal amplification. Moreover, one MNAzyme can cleave multiple substrate molecules resulting in signal amplification.

Finally, the CRISPR-Cas13 technology used in the concept entails a ribonucleoprotein (RNP) complex of the LwaCas13a enzyme and a CRISPR RNA (crRNA) that guides Cas13 to its ssRNA target. Target recognition and binding by the CRISPR-Cas13 RNP is based on sequence complementarity and is followed by activation of CRISPR-Cas13's endonuclease activity and subsequent collateral cleavage activity of RNAs in its vicinity. The flexibility of working with a DNA-based sensing method allows for a simple integration of all parts, eventually leading to a signal amplification cascade. The ssRNA product of the cleaved molecular beacon serves as a trigger of the CRISPR-Cas13 complex and its cleavage activity. As a result, Cas13 starts cleaving the reporter molecule, which is an ssRNA with a fluorophore and a quencher on each end. Over time, this, in combination with the MNAzymes cleavage activity, will result in enhanced signal amplification.

Cartridge Technology



The bioassay is implemented on a PVC cartridge. It is made out of four 300 µm thick PVC sheets interleaved with double sided adhesive tape as illustrated in Figure 2. Electromagnets are used to manipulate the functionalized magnetic beads necessary for the bioassay cascade. In the first reservoir, the beads are mixed with the saliva to deplete the target and activate the MNAzymes.

Figure 2. A) *Schematic of the multi-layered PVC sheets cartridge design;* ***B)*** *Cartridge made out of PVC*

Consequently, the magnets are used to transport the beads to the second reservoir where they are mixed again so that the molecular beacons are cleaved, and a signal is generated. For the mixing, three electromagnets are activated sequentially (their positioning is shown on Figure 3). More than one magnet is needed for effective mixing, as switching of the polarity of one electromagnet is not sufficient, the internal magnetization of the beads efficiently follows the external field. A setup of three electromagnets arranged triangularly is used. Christabel *et al. (2015)* proved that this greatly improves mixing efficiency.

Physical Transduction

As mentioned above, the second reservoir contains a molecular beacon. Upon cleavage by the MNAzyme, the fluorophore dissociates from the quencher and the fluorescent signal is generated. Its intensity is proportional to the amount of the functionalized MNAzyme, which correlates with the amount of H1 present in the sample. Since the FAM label generates a signal by excitation at 485 nm, a blue LED (of wavelength around 450-500 nm) was used. For the detection of the generated signal, a longpass filter was connected to the smartphone. The microfluidic cartridge was placed in a dark, light-impermeable docking device similar to Figure 4B and a blue LED was positioned under the cartridge.

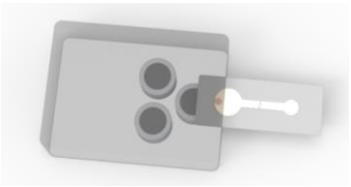
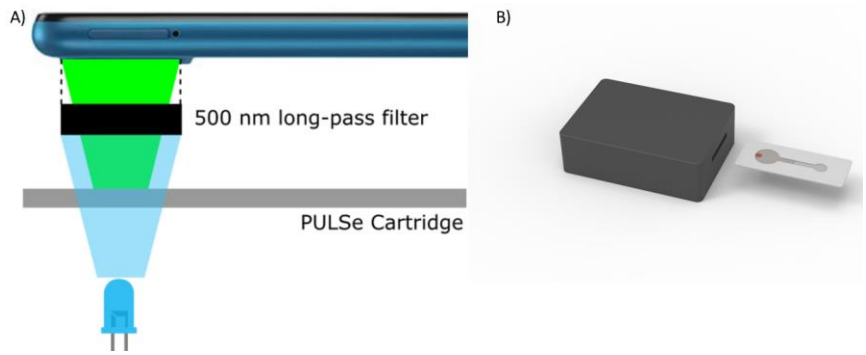


Figure 3. *The layout of the electromagnets used for electromagnetic mixing*

A 500 nm longpass filter is placed between the cartridge and the camera, as illustrated on Figure 4A. In this way, the background signal is minimized. Image processing then returns the intensity of the fluorescent signal.

Reader Instrument and User Interaction



The PULSe Platform consists of a docking device, on which a smartphone is placed. The cartridge is inserted into the docking device and slid by the user over a set of magnets needed for mixing and transportation. Once this transfer has happened, the readout of the fluorescent signal is performed with the smartphone.

Figure 4. A) *Optics setup for signal transduction* ***B)*** *A render of the docking device.*

The signal processing takes place on a remote server, making the platform easily expandable to other operating systems. The user needs to load the sample onto the cartridge, slide the chip in according to the instructions displayed on the smartphone, and wait for the result. Appendix 1 shows a view of the complete concept.

Technological Feasibility

Molecular recognition with aptamer

An ssDNA aptamer, V46, developed by J. Bhardway *et. al.*, has been selected for the recognition of the H1 stem region of the H1N1 surface glycoprotein (J. Bhardwaj *et.al*., 2019). FO-SPR was used to test the feasibility of H1 detection, functionalizing the optical fiber surface with the aptamer (A. Dillen *et al.*, 2021). Non-specific binding in pure saliva without the H1 target was tested for and no signal shift was observed. Meanwhile, a sample of H1-protein (10 µM) spiked in the buffer, showed a wavelength shift of 3.64 nm (Figure 5A). This observation suggests that saliva does not contain components binding to the aptamer in a nonspecific manner, which demonstrates the specificity of the selected aptamer to H1.

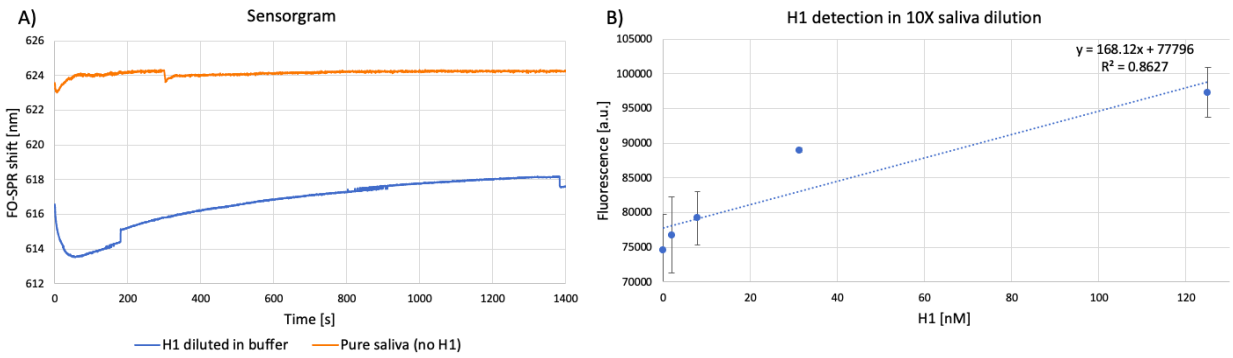


Figure 5. A) *Sensorgram displaying the FO-SPR shift for pure saliva without H1-protein and H1-protein spiked in the buffer.* ***B)*** *Dose response calibration curve of H1-protein. Error bars represent the standard deviation of three repetitions.*

In order to analyze the binding behavior of H1 to the aptamer hybridized with the inhibitor, a fluorescent tagged aptamer and a quencher tagged inhibitor were used. Once H1 binds to the aptamer, the corresponding inhibitor is released resulting in fluorescent signal. Measurements were carried out with a spectrophotometer to build a calibration curve with a series of H1 concentrations (Figure 5B). A positive linear correlation is observed between H1 concentrations and the elicited fluorescence, which coincides with the binding of H1 to the aptamer.

Signal conversion with MNAzyme

The MNAzymes were immobilised on magnetic beads with a streptavidin-biotin chemistry. In order to verify whether the immobilisation hindered the catalytic activity of the MNAzymes, the functionalized beads were tested by adding various concentrations of the facilitator and either substrate or molecular beacon, where the former served as a validation reference (Figure 6).

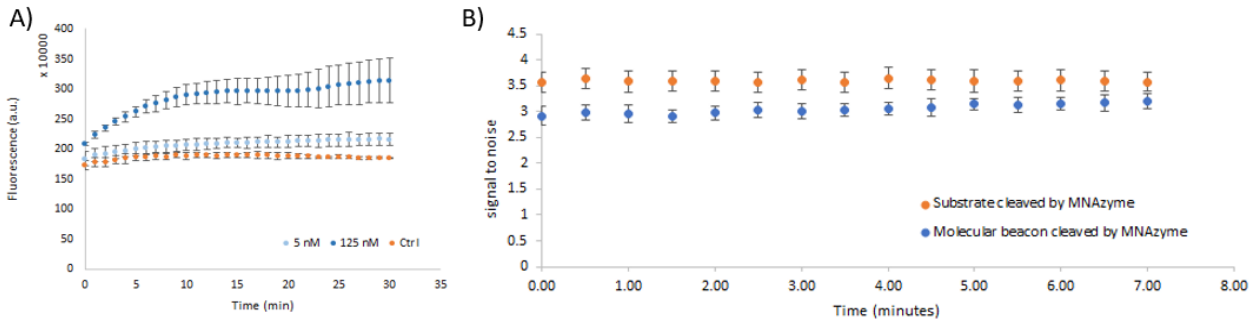


Figure 6. A) *Cleavage of substrate by immobilised MNAzymes.* ***B)*** *Comparison of the reaction with molecular beacon and with substrate. Error bars represent the standard deviation of three repetitions.*

Validation of the assay was done by measuring the fluorescence generated upon cleavage of the fluorophore- and quencher-labelled substrate or molecular beacon, with a spectrophotometer (Figure 6AB). Measurements were carried out in 384-well plates and with trifold repetitions. Excitation occurred at 485 nm while the emission wavelength was 535 nm. Finally, the MNAzymes were tested in combination with the molecular beacon to see whether the beacon can be cleaved by the MNAzymes. This verification happened in solution rather than on beads. It was opted to use a signal to noise ratio to make the comparison due to the difference in raw fluorescence signal between the substrate and the beacon. This way ratios of both the beacon and substrate can be compared to determine whether there is a significant difference. Based on the data, it can be concluded that the MNAzymes can still cleave the beacon since the signal to noise ratio is higher than 1. Moreover, the signal to noise ratios are in a comparable magnitude.

Signal amplification with CRISPR-Cas13 technology

The finalized product could not be reached within the timeframe of the competition. However, there are multiple elements supporting the feasibility of LwaCas13a for signal amplification. Literature reports a LwaCas13a based nucleic acid detection platform known as specific high-sensitivity enzymatic reporter unlocking, SHERLOCK, (Jonathan S. Gootenberg, *et.al.*, 2018). It demonstrates that a 0.5 pM concentration of ssRNA can be detected purely through Cas13's collateral cleavage activity. Based on the SHERLOCK technology, DISCoVER demonstrated a CRISPR-Cas13 based diagnostic assay that enabled Sars-CoV-2 detection in saliva within five minutes (S. Agrawal *et.al*., 2020). In order to estimate the potential of a CRISPR-Cas13 based readout, calculations were obtained from in-house developed models of MNAzyme kinetics. For every H1-protein that binds to the aptamer, the corresponding inhibitor strand is released, resulting in binding and activation of one MNAzyme. A concentration of 0.01 pM of MNAzyme cleaves 0.68 pM of molecular beacon resulting in 0.68 pM ssRNA substrate to activate CRISPR-Cas13. This coincides with the detection sensitivity previously reported by SHERLOCK.

Fluidic Cartridge

Autofluorescence of the used materials should be avoided to minimize background signal. Therefore, the autofluorescence of the cartridge made out of PVC and PSA was investigated and a black PMMA mask that covered the entire cartridge except the readout reservoir was used to eliminate reflection of light.

The strength of the electromagnetic field was experimentally verified and showed that small permanent magnets were sufficient to manipulate the beads. The magnetic field flux of this permanent magnet was measured to be 115 mT. The requirements of the electromagnets could be calculated from this. Using a 10 mA current means 14 coils are required for a 5 cm electromagnet. Since the Arduino we used has a 5V output signal, a 500 Ohm resistor should be added in series with the magnet to achieve the desired current of 10 mA. This 5V output signal is also similar to typical smartphone port output values proving that it is feasible to use the smartphone to power the docking device.

It was estimated how many beads would be needed to deplete the sample. For this, it was assumed that the number of magnetic beads needed is a factor 1000 higher than the maximum number of target proteins that has to be detected. This corresponds to 10^{13} particles. Converting this to a concentration for a 100 µL volume means that the beads should have a concentration in the micromolar range.

Adaptation for Influenza A Readout

The biosensor makes use of a ssDNA aptamer, V46, to target H1. Jyoti Bhardwa *et al.* demonstrated that V46 has the ability of targeting both H1 isolated particles as well as the H1 surface glycoprotein on the whole virus's envelope (Jyoti Bhardwa *et al.*, 2019), meaning the aptamer would be able to bind to the H1 viral particle on the whole virus and no steric hindrance would impair the specific binding. Hence, the aptamer-H1 detection would not require major adaptations for the measurements of intact influenza A virus. Nevertheless, one would need to adapt the calibration curve in accordance with the intact virion's H1 concentration.

Originality

Written by Team Captains

Over the past few decades, biomedical sensing tools have gained a colossal interest. This mostly coincides with the evolution of detection technologies. Biosensors have become irreplaceable cutting edge analytical devices because of their high sensibility, sensitivity, and reliability (Anas Islam *et.al*.,2021). Transducing a chemical signal in a fluorescent signal in a sensitive way can be complex. However, PULSe's novel and highly innovative biosensing concept has met this challenge. Aptamer, MNAzyme or Crispr-Cas based biosensors have been supported and shown promise in literature (Wei Diao et al.,2016, Wenwen et al., 2020, Richard Bruch, 2019, Andrea Bonini *et.al*., 2020). However, to our knowledge, the concept we propose is unprecedented. Major innovations include (a) the combination of the aptamer, MNAzyme, molecular beacon and LwaCas13a to enable highly sensitive biosensing to the picomolar range (b) the proposed microfluidic concept allowing the segregation of activated MNAzymes from the initial salivary solution through (i)SIMPLE chip and presence of electromagnets as a way to displace particles.

As a team, we started our work by reading up on important aspects of hemagglutinin, different possibilities of detecting low concentrations of a protein, working with complex matrices such as saliva and similar problems associated with the challenge at hand. Over the span of the first three months, with guidance of our supervisor and coaches, we have narrowed down the ideas of interest to the most viable ones, taking into account the knowledge and technologies available in the MeBioS lab. Eventually, this led to a concept that would be implemented and perfected for the purpose of the competition and from that point on, we worked in the lab with this aim. We planned and designed our experiments mostly independently, with advice from the coaches during regular update meetings, where we also assured collaboration between the bioassay, sensor tech and business subteams.

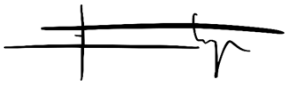
Our ambition has caught up with us as we could not achieve the final product within the time frame of the competition. The present biosensor concept feasibility is backed by extensive literature research and results of preliminary experiments we have performed.

Written by Supervisors

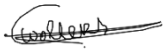
The team received support from us, the supervisor and coaches, in every step of the development of this biosensing concept. More specifically, in the initial phase of the project, we discussed a selection of technologies and biosensing concepts with the team, inspired by the expertise and materials available within our group. Next, based on these concepts, an extensive literature search, and their own interests and knowledge, the team came up with a number of ideas for the biosensor. Subsequently, we guided them in deciding on the final biosensing and readout principle, to end up with both a novel and feasible biosensing concept. 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.

The business plan related to this biosensing concept was fully developed by both the students and the supervisors. The students looked independently for relevant information and had interesting discussions with sponsors and experts in the field of health and prevention that were crucial in preparing and validating their business strategy.

Supervisor: Jeroen Lammertyn

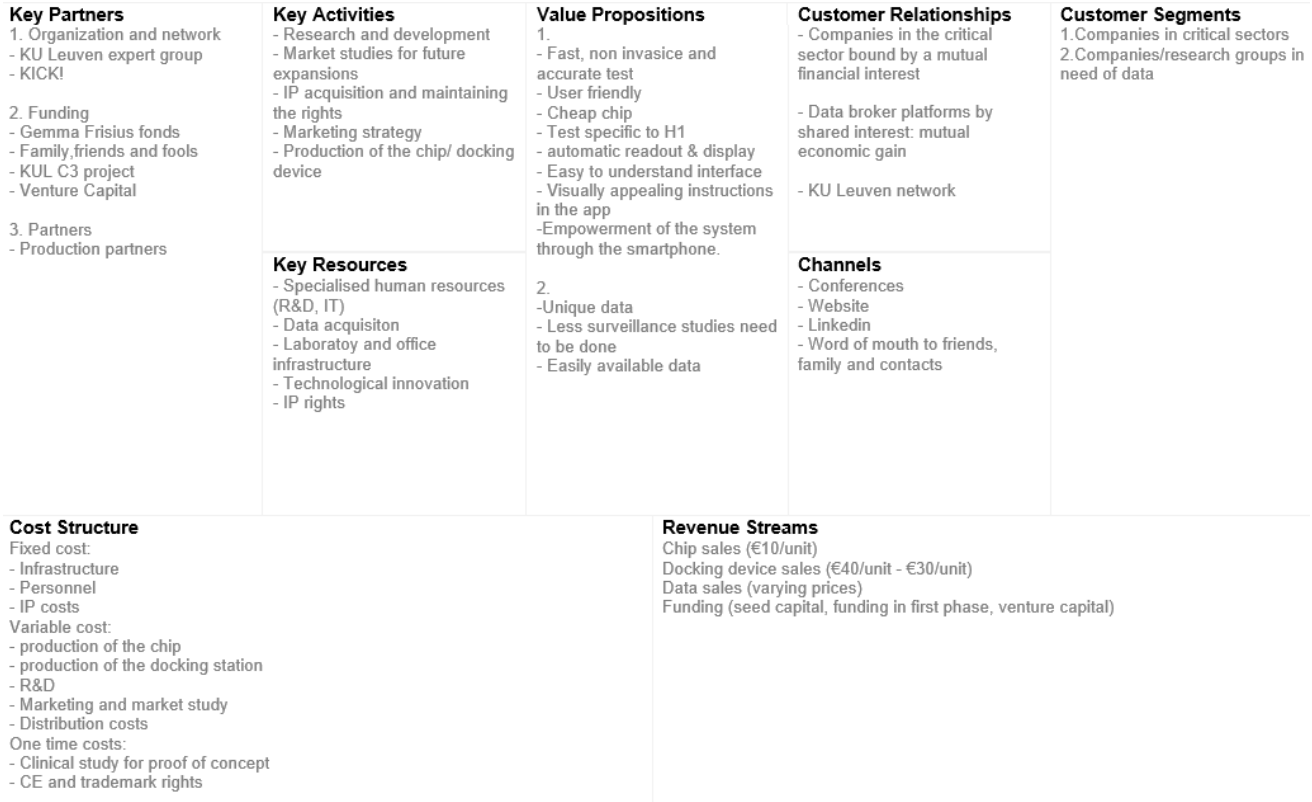


Team Captains: Eléonore Wolters & Darina Abaffyová



Translational Potential

Business Model Canvas



Market Description

To obtain a clear overview of the market, it is essential to define drivers of seasonal flu epidemics: (air) traveling generously contributes to global spread of influenza A, whereas via schools young children typically push the ball downstream to their families (Kenah, E. et al. 2011, Coleman, K. et al. 2020). In turn, the majority of the family members regroup in new clusters at work and enforce divergence of the spreading pathway to other co-workers as a result. For this reason, PULSe aims to dampen the spread of this respiratory virus at work environments, by on-the-job decentralized testing, and will initiate the introduction of our biosensor on the essential sectors' market in phase I. We are keeping in mind that these critical functions ought to be the motor behind the continuity of society, accounting for a market size of 693,700 patients. Employees in critical functions have irreplaceable technical expertise and require utmost attentiveness to succeed at performing their job. An overview of these sectors can be seen in Appendix 5. Companies with a critical function can be defined as those which experience a huge impact when their employees are less productive or sick. Taking Belgian ports as a prime example for these types of companies. Approximately 11 100 people work there and 1825 companies are dependent on the ports. Any disturbance to the workflow of port workers will result in substantial economic consequences. As a consequence any precaution that can guarantee the continuity of the company should be adapted. Moreover, these types of companies are present in most countries making internationalisation in later stages possible. We substantiate it by our example as the Belgian ports serve as a distribution centre towards Western Europe as a whole.The reasoning for choosing these critical companies is because employees carry more responsibilities and hence are more willing to self-test.

With the technology of our biosensor, an asymptomatic patient that tests positive will be able to have their diagnosis confirmed at work within five minutes and will make the currently occuring step to the GP for medical proof obsolete. Secondly, we shift the paradigm to testing preventively in the asymptomatic state to enable earlier source control in these

companies: before employees start working, they get tested non-invasively to start their day or will have to take necessary precautions and isolate.

Stakeholder Desirability

Influenza annually infects 5-15% of the population in the northern hemisphere, with the possibility to increase up to 20% during peak months if left unattended. (WHO (2021)) The pathogen has a seasonal character reaching its peak in the winter months. Moreover, in tropical areas it is common to have influenza activity all year-round. (WHO (2021)) Seasonal flu brings about 2 and 5 million cases with serious symptoms annually. (WHO (2021))

Furthermore, influenza also has a socio-economic impact. It's responsible for big financial losses. These are caused by hospitalisation, absenteeism for disease recovery or by having to take care of family members (Jeffrey et al, 2017). However, the biggest financial loss is sustained by the employers in the shape of loss in production due to sick, or less productive employees. (Uhart et al, 2016) Through simulations and calculations, it was estimated that influenza can decrease the GDP by 0.3% to 3.7% depending on how infectious the specific viral strain is. (Smith et al, 2011). These percentages were used to estimate which losses would be led by a company during a regular flu season. For the design of this business case, it was opted to use a more negative scenario,meaning the viral strain would not be very infectious. Such a flu season would correspond to approximately 10% of employees getting sick resulting in a loss of 1% revenue to the companies on average. This risk is especially relevant for companies in which limited to no replacement is possible.

In addition, in-vitro testing at the Doctor's office consists of sample collection and PCR processing by technicians in a lab. Although it is much more sensitive and faster in comparison to the gold standard (cell culture) (AHS 2021), it is not cost-effective and the time-to-result is not in line with the duration of consultations. On the other side, rapid influenza diagnostic tests are not considered sensitive enough with the use of antibodies for detection (AHS 2021). Our biosensor covers all beneficial aspects of the PCR tests, while it does not require the involvement of the lab technicians and doctors. The biosensor is also valuable in asymptomatic patients and prevention.

Our business strategy involves multiple stakeholders that are divided in a phased approach. In the first six years, companies with a core function in society and economy will be important as they serve as our entry to the market.

Data broker platforms are the second stakeholder. In the primary phases of the business plan they will only play a minor role, since the sensor will only generate a limited amount of data. In later phases these stakeholders will only become more important due to the increase in processed data. The data itself can be considered as an additive value being created.

Business Feasibility

The PULSe start-up consists of a technical team composed of a bioassay team, a microfluidics engineer and a non-technical manager located in Bio-Incubator, Leuven. It is assisted by an advisory board with a diverse background: members from MeBioS KU Leuven who are specialized in biosensor technology and microfluidics and Professor Bart Preneel who created the Coronalert mobile application which was developed for the management of the coronavirus pandemic. His expertise will help with the development of the smartphone application, creation of a database, data management and data security.The biosensor will evolve through different stages starting from the prototype.

Phase I: Initialisation (2021-2023) - During this phase R&D and finalisation of the concept are realised. Subsequently, the mobile application and biosensor are validated and subjected to clinical trials.. After approval, targeting companies within the portfolio of a critical sector starts. A stable 1.5% of the market should be achieved. In this phase data is sold at a lower price to improve visibility of the product.
Phase II: Connecting the market (2024) - It is aimed to increase the market share, an increase from 1.5% to 5% in market share should be accomplished. In this stage, data continues to be sold but the price increases.
Phase III: Growth (2025-2027) - Addition of The Netherlands and Luxembourg will occur ensuring PULSe's foothold in the Benelux. Market shares are aimed to stabilize at 5%. During this stage, data sales will become increasingly important as its

volume increases, data prices will increase. This phase will span multiple years ending in 2027. Every year will be characterized by a 2.5% market share increase which will result in 10% by 2027.

To successfully commercialise our device, smart marketing of our value proposition will prove essential. Moreover, collaborations with data brokers will become increasingly important. Due to the adaptability of our assay, continued research will target future opportunities.

Financial Viability

PULSe's financial viability is supported by a novel business strategy. Our revenue stream consists of 3 main points: income from selling cartridges, the sale of data and revenue from selling the docking devices. The sale of the cartridge and data will be key drivers for Pulse's financial growth. The cartridges will be sold for €10/unit while the initial price of the docking device will be €40/unit. By 2027 the price of the docking device will decrease to €30/unit. The price of data will vary based on the size and history of the dataset. At the start data will be priced at €0.65 per record to increase attention for our data. Based on the increase in volume of the data it will reach a final price of €0.90 per record by 2027. Given the variable character of the seasonal flu, it was opted to do a worst case scenario financial analysis. Our market consists of approx. 693 700 employees in a critical sector in Belgium, of which we should target 1.5% in 2023. By 2024 this market share should increase to 5%. We would like to continue this trend in the Benelux where the addressable market consists of 1966589 people, meaning approximately 108 600 customers by 2025. Afterwards the market share in the Benelux should increase to 7.5% and 10% by 2026 and 2027 respectively. A fully detailed calculation can be found in the appendices, while a summary can be found in Figure 7 and Appendix 6. Prior to commercialisation in 2023 there are 2 years of R&D and optimisation. The break-even point will be reached between 2025 and 2026, five years after starting development.

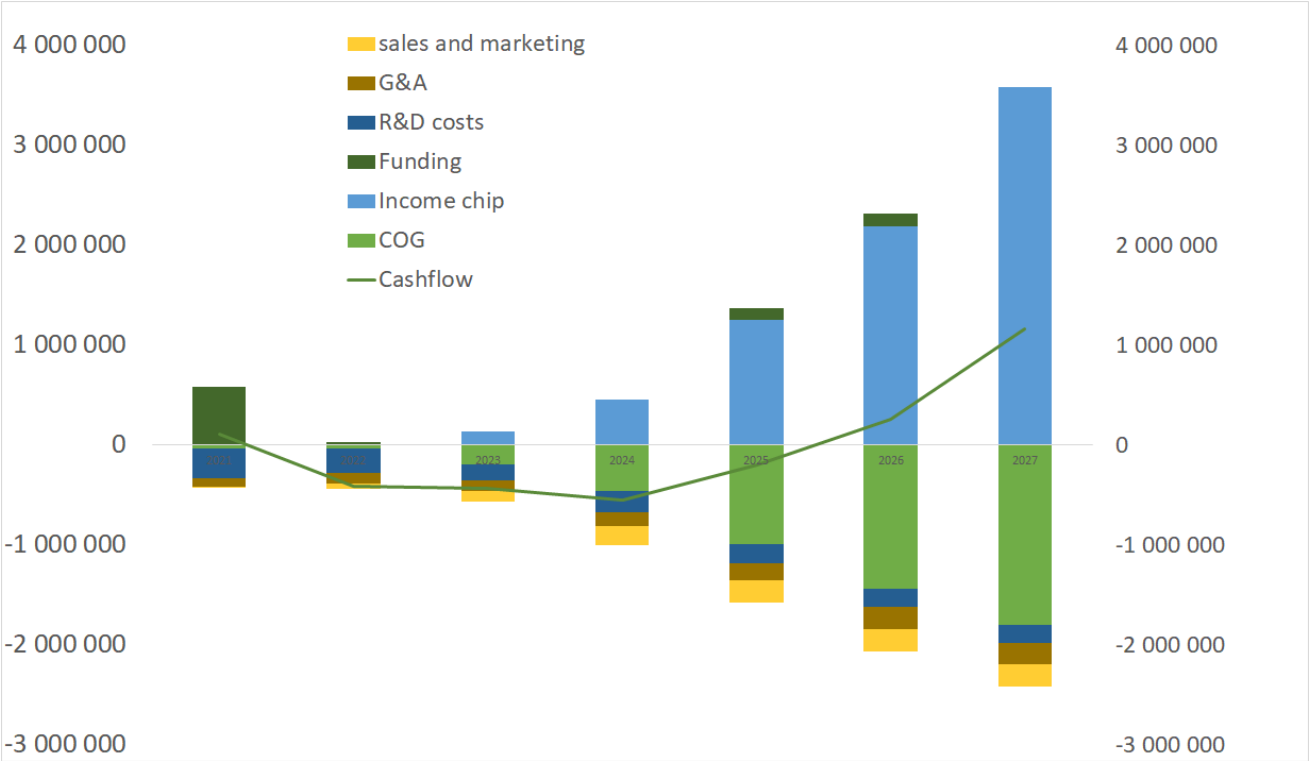


***Figure 7**. Estimated revenue, cost projection and net income for the first years of operation*

Team and Support

Contributions of the Team Members (more in Appendix 2)

Darina Abaffyová - Team captain of the sensor technology team and member of the communication subteam.
Jintong Ge - Member of the sensor technology and communication subteams.
Anaïs Hubart - Member of the bioassay, sponsor and business subteams.
Ivana Janíčková - Member of the sensor technology and sponsor subteams.
Maxim Lambrechts - Member of the bioassay development and the business subteams.
Evelyn Lerinckx - Involved in the bioassay development subteam as well as the business subteam.
Lai-Jin Lily Leung - Member of the bioassay development team and became the team leader of the business team.
Vasilis Loukopoulos - Member of the sensor technology and business subteams.
Wout Mens - Member of sensor technology and the sponsor subteams.
Emily Rosschaert - Member of both the bioassay and the sponsor subteams.
Eléonore Wolters - Team captain of the bioassay development team and member of the communication subteam.

People Who Have Given Support (more in Appendix 3)

Prof. Jeroen Lammertyn, founder of the MeBioS Biosensors research group. Without his guidance PULSe could not have participated in the SensUs competition. Thanks to his generosity the team could make use of his labs with the appropriate equipment and support.

All of the coaches were PhD candidates in the MeBioS- Biosensors group of Prof. Lammertyn. Their expertise, knowledge but also their pedagogical capacities and moral support were crucial for the team. **Aida Monserrat Pagès, Yagmur Yıldızhan** and **Charlotte van Tricht** were specifically involved in the bioassay development subteam while **Lorenz Van Hileghem** and **Wannes Verbist** coached the sensor technology development subteam. Words could not thank them enough as they have been present and available to answer our doubts and questions at any time during the entire competition. Importantly, they also ensured a good team cohesion. **Charlotte van Tricht** and **Lorenz Van Hileghem** additionally gave precious support and advice to the business team.

Lastly, we would like to highlight the support received from **Annelies Dillen,** regarding the Aptamer Complement Element, **Ruben Cops** for the safety rules and regulation training at our lab and **Dr. Dragana Spasic** for giving proper feedback on the biosensor concept.

Sponsors

We want to thank our sponsors; **Antelope Dx, FOx Biosystems and Waters Corporation** for their financial support and the invaluable advice offered through their knowledge and expertise. We explicitly want to thank our Platinum sponsor, **Comate Engineering**, for supporting us with their advice throughout the whole process and the development of animations for our pitches.

Final remarks

Over the past nine months, we have learned not only about the technical and business related aspects of what it takes to develop a biosensor, but we have also improved our soft skills—efficient time management, communication on different levels of expertise and effective interdisciplinary team work, to name a few. We want to thank SensUs for organising the competition and to KU Leuven for taking part in it, which provided us with this great opportunity. A big thank you goes to our coaches, for the time and effort they invested into leading us throughout this experience. For their invaluable advice and financial support, we thank all of our sponsors. Last but not least, it goes without saying that without the contributions of each and every one of the team members the project would have never reached its final form.

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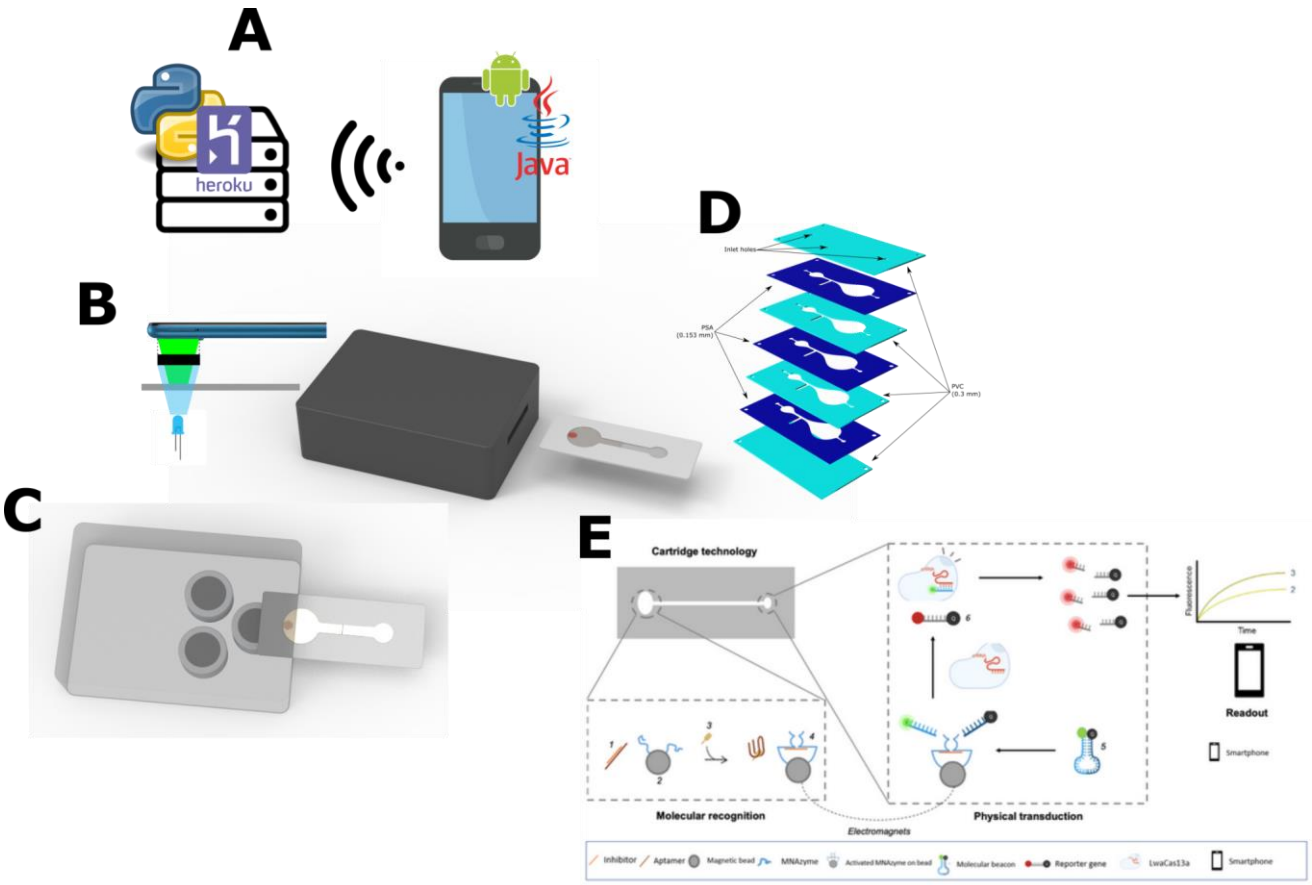
Appendices

Appendix 1: Complete Concept



The smartphone would be positioned on top of the docking device (i.e. the black box in the centre).
A = Communication of the smartphone device with signal processing implemented on a server (Heroku was used for our prototyping purposes).
B = Optics of the system.
C = Magnetic beads.
D = PULSe cartridge.
E = Bioassay concept implemented on the PULSe cartridge.

Appendix 2: Contributions of the Team Members

Darina Abaffyová was one of the team captains and member of the sensor technology and communication subteams. On top of work on the readout, she took responsibility for making sure all deadlines were met and relayed communication from the organisation to the team.

Jintong Ge was a member of the sensor technology and communication subteams. In the sensor technology team, she worked in the lab, analyzed data and adjusted the parameters of the set up. In the communication team, she helped manage PULSe's social media.

Anaïs Hubart was in the bioassay, sponsor and business subteams. From the start, she has been dedicated to each of these three teams. Focussing on the MNAzyme aspect of the concept, through the business plan to the sponsoring brochure, her work was substantial.

Ivana Janíčková, as a member of the sensor technology and sponsor subteams, was contributing not only in the cartridge development and readout of the signal, but also helped with recruiting and communication with our sponsors.

Maxim Lambrechts was a pivotal member of the bioassay development and the business subteams. He was a fixed value during the whole competition, spending an incredible amount of hours in the lab doing experiments, data analysis and working for the business team.

Evelyn Lerinckx was involved in the bioassay development subteam as well as the business subteam. She contributed to the MNAzyme aspect of the bioassay and was the initial team leader of the business team.

Lai-Jin Lily Leung was an integral member of the bioassay development team and became the team leader of the business team. She devoted her time to the aptamer aspect of the bioassay and her dedication and contribution to the business team was invaluable.

Vasilis Loukopoulos, was a member of the sensor technology and business subteams. He focused on the cartridge development and manipulation of the magnetic beads, while contributing to the business team.

Wout Mens was in the sensor technology and the sponsor subteams. He contributed significantly to different aspects of the readout and manipulation of magnetic beads and helped with recruitment and communication with our sponsors.

Emily Rosschaert was an essential member of both the bioassay and the sponsor subteams. Her contribution to the aptamer aspect of the bioassay, the sponsoring's brochure and the communication with the sponsors was more than precious.

Eléonore Wolters was one of the team captains and member of the bioassay and communication subteams. Her work on the aptamer portion of the concept was indispensable, and her leadership skills were valuable for the management of the team.

Appendix 3: People who have given support

Aida Monserrat Pagès was a pivotal coach in the bioassay development team. She coordinated the MNAzyme and aptamer aspects and made sure to take time to explain any unclarity to the team members. Her data analysis and pedagogue skills greatly helped the team members in understanding and precious time saving.

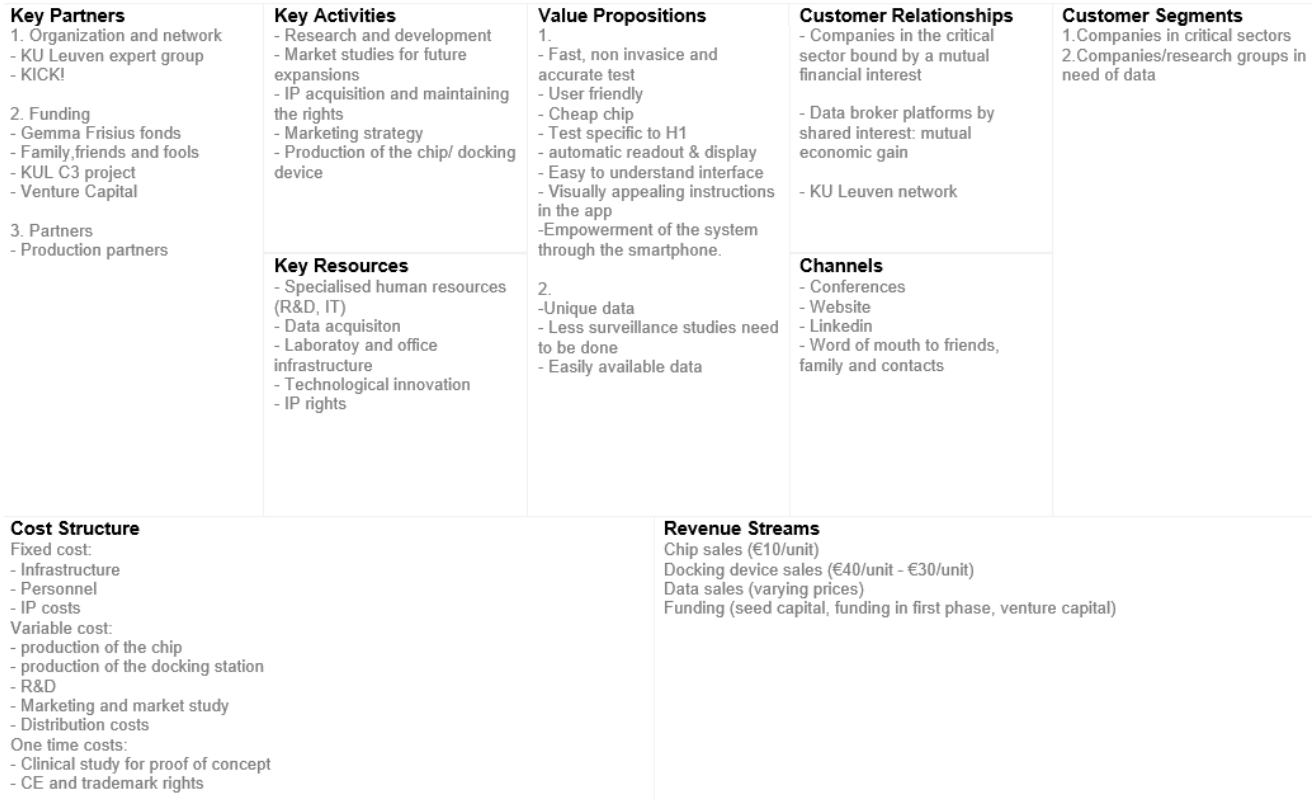
Lorenz Van Hileghem coached the sensor technology and business subteams. His contribution and guidance in both teams were essential.

Charlotte van Tricht coached the bioassay development and the business subteam. Her help and input was precious all along the way.

Wannes Verbist was involved in the sensor technology subteam, where he readily provided valuable advice and feedback when necessary.

Yagmur Yıldızhan, PhD student in the MeBioS-Biosensors group. She coached the bioassay development team. The aptamer team could not have made it without her expertise in FO-SPR.

Appendix 4: Business model canvas



Appendix 5: *market of essential sectors, phase I*

Sector	**Approx. Amount of employees**
Petrochemical	59400
Ports of Belgium	11 100
Supermarkets	155 000
Food industry	96 000
Agriculture	196 000
Electricity plants	11 400
Air traffic control	800
Teachers	164 000
Total	**693700**

Appendix 6: Detailed cost analysis