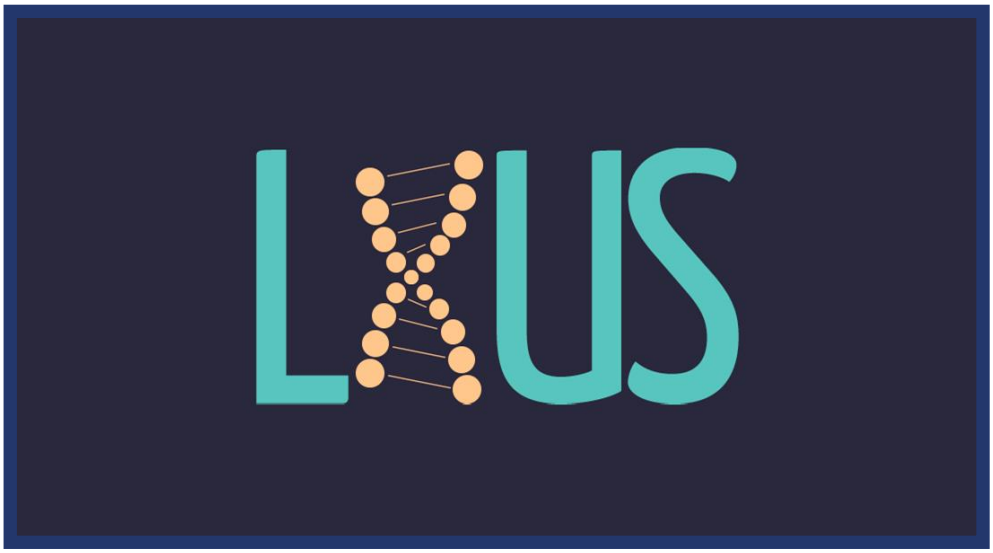
Team Results Document

LxUs



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SensUs

LISBOA | UNIVERSIDADE DE LISBOA

2021
Influenza

Abstract

Usually, the flu has a bigger impact on elderly people. In 2019, Portugal registered 19.5% cases of flu in people over 65 years old (INSA). The current pandemic has led to the use of masks that has significantly reduced the number of cases of flu, yet the problem has not disappeared. There are 29 Influenza A viruses subtypes detected until now (CDC). Rapid and reliable testing is essential to fight pandemics.

With the new paradigm, industries, companies, and people adapted. For instance, pharmacies became a testing hotspot. Given that is not their focus, it required fast and efficient change to serve the community. This reduces hospital pressure. Our biosensor would be placed in the pharmacies, requiring little to no intervention of the pharmacists and adding a new revenue stream.

Optical methods to detect biomolecular interactions enable label-free detection and have lower noise levels. We chose Surface Plasmon Resonance (SPR) for detection. The SPR equipment is very expensive, so our challenge is to develop a cheaper apparatus while maintaining the maximum sensitivity possible in accordance with the Goal 3 and 10 of the UN of promoting well-being while reducing inequality among countries by providing cheap and reliable testing equipment.

Biosensor system and assay

Optical biosensors enable label-free detection and have high specificity and sensitivity (Svitel, 2016) which is crucial to track the propagation of a virus and prevent a possible pandemic. During our literature review we found several optical methods, **Surface Plasmon Resonance (SPR)** was the one that stood the most for us, given that it does not consume reagents continuously such as for instance enzyme-linked immunosorbent assays (Naimushin, 2002). Additionally, SPR has multiple advantages such as real-time data monitoring, being a label-free method and being highly adaptable (Tang, 2010; Suenaga, 2012).

The **physical concept of the SPR** phenomenon is achieved through the existence of a light source, a prism, a metal film (usually a gold film) and a detector – Appendix A, Fig. A1. The film is placed strategically between two different dielectric media, the prism (medium 1), with a higher refractive index, n_1, and the solution under analysis (medium 2), which has a lower refractive index, n_2 (Tang, 2010). The light beam is emitted from the source and passes through the prism, which is reflected to the detector at a certain angle of incidence, known as the resonance angle. Eventually, the light is absorbed by the electrons present in the gold film and consequently causes them to resonate. These electrons are **extremely sensitive to any change of the adsorption of molecules** on the surface. Thus, there is a loss of intensity of the light beam being reflected.

The **transduction** itself is achieved by combining a laser, an oscillating reflective surface such as a mirror to generate the angular sweeps, and a receptor, such as a photodiode. This last instrument is used to **measure the number of photons hitting the detector area**, which in turn creates a current proportional to the number of photon detections. This current can then be converted into a usable reading that tells us how intense the reflection is. By **detecting a drop in intensity**, we can verify that **the active surface is interacting with hemagglutinin**, and therefore the sample provided contains Influenza. There are several factors that might **create artefacts in experimental results**. Since SPR detects the change in refractive index of a liquid, in this case the saliva, the influence of temperature should not be negligible. Thus, we need to create a detection unit which is temperature controlled. The idealized temperature is obtained after running several experiments to study the thermodynamics of the binding.

Thus, the **molecular assay principle** chosen was the **antigen-antibody interaction** since this is a very specific interaction that will bring a higher sensitivity to our biosensor. For that purpose, we need a surface where we have the antibodies immobilized. The **gold surface** has unique optical properties for the SPR **providing reproducibility** to be easily modified. This surface is also chemically inert to solutions and solutes and is commonly used in biochemical context (SPRPages, 2021, July 31). To increase the efficiency of SPR technique, the gold surface is required to be rough and $30 - 50\ \mathrm{nm}$ thick.

Consequently, the **immobilization surface** consists of a **gold surface** with a **polydopamine-ethanolamine (PDA-ETA) layer** (Almeida, L. C., 2021) – Appendix A, Fig. A3. Using PDA-ETA, we will increase the roughness of the surface and provide a better distribution of the antibodies, which will result in better experimental results. Moreover, **ethanolamine** functions as a blocking agent such as BSA, since it blocks non-specific interactions and, as a result, increases the sensitivity of the biosensor. **Protein A** is added because it allows greater exposure of the antibody binding sites and reduces ligand heterogeneity that happens during immobilization. The schematic of the immobilized surface with the SPR and cartridge is represented in Appendix A, Figure A4.

To **minimize matrix effects**, known as the changes in the extension of the dextran matrix on the sensor chip surface due to variations in pH or ionic strength, we match the injected sample to the flow buffer, **Phosphate-buffered saline (PBS)**. The ligand density in the immobilization surface should be the lowest possible. However, when the ligand densities are low, noise and drift are facts that we should take into consideration, so it is necessary to equilibrate the sensor surface and carefully match the flow and the buffer.

For the **molecular recognition** of the Influenza A H1N1 (A/California/04/2009) Hemagglutinin/HA, we chose to use **Influenza A H1N1 (A/California/04/2009) Hemagglutinin/HA polyclonal antibodies** produced in rabbits immunized with purified, recombinant Influenza A H1N1 (A/California/04/2009) Hemagglutinin/HA. For the immobilization, the sPBS buffer ($\mathrm{pH} = 7.4$) is always used during the assay.

SPR is also extremely adaptable, for instance there are several articles **with SPR build-in a smartphone** (Lundström 2014; Bremer 2015; Liu 2015; Guner 2017; Quesada-González 2017). However, these systems do not report the same sensitivity as commercially available SPR instrumentation (from Biacore and OpenSPR), since they are phone model specific and thus, not appropriate for large-scale implementation (Preechaburana, 2014). Usually **SPR instrumentation is quite expensive**, ranging from € 50,000 to € 300,000. This range of price is justified by the small

number of companies that fabricate SPR devices and can be justified by the impact of technological progress that follows supply and demand (Appendix B). We believe that SPR is still in the early adoption phase and in a niche market. However, the choice of SPR allows **for adaptability of the chosen immobilization surface**. Our biosensor can detect other respiratory diseases (such as COVID-19) or other biomolecules by changing only the antibodies and thus the binding interaction.

The **cartridge technology** was designed with **ease of use** in mind but also to make it **easy to produce and distribute**. As we are employing SPR technology, which typically handles samples with a **microfluidics system**, our first approach was to try to integrate such a system, using pumps, valves, filters, and mixers to manipulate the saliva sample. This proved to increase the complexity of the equipment as not only it would require more space and power, but also requires a higher fabrication cost, as microfluidics chips require specialized fabrication techniques. This would also pose problems regarding the collecting of the saliva sample itself as the collecting and the analyses of the sample would have to be done in two very distinct steps. Thus, we needed to implement our own approach that wouldn't require such components. Our aim is to make the analysis of the saliva sample a **simple process**, from the perspective of user experience, but also to facilitate the saliva collection step and at the same time **using cheaper and easier fabrication techniques**.

We investigated common methods of saliva collection and commercially available solutions. We reached the conclusion that the **passive drooling method** was the most applicable for saliva samples as it provides the **collection of saliva in a short time**, while **minimizing potential sources of error** (Bhattarai, 2018). It also proved to be the best method for our design as it is a simple process because it doesn't involve so many resources (material and human) in comparison to the other options considered.

Furthermore, our solution for the cartridge technology involves two components that come together. The first one is the saliva collector which consists of a **simple Eppendorf** (Appendix A, Fig. A2) with an insertable lid. To facilitate the flow of the saliva to the Eppendorf and to prevent contamination of the outer surface of the Eppendorf, we include a **hydrophilic paper tunnel** which should be placed on the open end of the Eppendorf and discarded after collecting the sample. The second component is the **cartridge itself which contains the gold surface**, with the immobilized antibodies, placed in a glass surface. Similarly to the microfluidics systems, this cartridge includes **a channel to redirect the saliva sample** from an inlet to the immobilized surface. The poster with instructions placed in the biosensor are in Appendix A – Figure A5.

To ensure that the card is correctly placed for the analysis, **two small magnets** are placed at the end of the card. Thus, whenever the card reaches the end of the slot, it connects to two other magnets placed inside the equipment. This can be used in combination with a magnetic sensor to **detect that the card is indeed placed** and **ready to start the analysis**, triggering the software to start its routines. Our aim is to carry out this process without involving great supervision and the least resources as possible. The procedure of the test requires the user to remove the Eppendorf lid, place a hydrophilic paper funnel inside the Eppendorf and place its head horizontally to the floor so the saliva passively drools to the inside of the Eppendorf until a mark of the optimal volume of saliva is reached.

Afterwards, the user takes the cartridge itself, rotates it, placing the x surface facing the floor places the entry on the Eppendorf open end, and places the cartridge over the Eppendorf until it hears a click that indicates the Eppendorf is attached to the card. Then the user has to rotate the card again, with the Eppendorf facing the ceiling and places it inside the slot. Much like an ATM, this triggers the equipment to start analysing the interaction between the sample and the antibodies while it asks for **the user's identification number and/or contact** (e-mail or phone number). After completing this step, the user is free to wait or leave as it will receive the result via the provided contact. When trying to expand the biosensor to our countries, we will take into consideration that not all countries have the same access to internet and smartphones, so we will adapt our forms of contacting accordingly or give the result in the spot.

Technological feasibility

The **SPR configuration** chosen is the **Otto configuration**, where the light is totally internally reflected, and the gold film is placed in contact with the prism (Ahn, 2018). This configuration is represented in Appendix A - Figure A6. The **resonance angle** ($S_{\theta,p}$) can be estimated using:

$$S_{\theta,p} = \frac{Re(\varepsilon_{metal})\sqrt{-Im(\varepsilon_{metal})}}{\{Re(\varepsilon_{metal}) + {n_{analyte}}^2\}\sqrt{Re(\varepsilon_{metal})\{{n_{analyte}}^2 - {n_{prism}}^2\} - {n_{prism}}^2 {n_{analyte}}^2}}$$

Where ε_{metal} is the metal permittivity, $n_{analyte}$ the refractive index of the analyte and n_{prism} is the refractive index of the prism. The **spectral wavelength** ($S_{\lambda,p}$) is determined by:

$$S_{\lambda,p} = \frac{Re(\varepsilon_{metal})^3}{\frac{{n_{analyte}}^3}{2}\left|\frac{d\,Re(\varepsilon_{metal})}{d\lambda}\right| + \{Re(\varepsilon_{metal}) + {n_{analyte}}^2\}Re(\varepsilon_{metal})\frac{d\,Re(n_{prism})}{d\lambda}\frac{n_{analyte}}{n_{prism}}}$$

Where the permittivity is wavelength-dependant, hence it is defined it as a complex number and is also a function of the loss angle (Wikipedia, 2021, July 25; RefractiveIndex.INFO, 2021, July 25; Landau, 2013). $\frac{d}{d\lambda}$ is defined as the chromatic dispersion and is usually defined in units of μm^{-1}.

With these estimates we can design our biosensor and better optimize for sweep ranges and where to better position the components and optics to enhance the performance of our design. We obtain **a parametric design** where the choice of analyte and parts such as prisms and its characteristics, making our design very simple to change, depending on a few predetermined parameters.

Before performing any test, we must **automatically calibrate the instrument** and **measure the baseline** to compare the results. Thus, the idealized SPR instrument has two channels: one for the baseline and other for the saliva sample. The light intensity measured with the photodiode will be a function of the angle of incidence – Figure A7 in Appendix A.

In practice, the signal measured will be the voltage generated by the photodiode. The voltage will depend on the concentration of antibodies that are bonded with the antigen. These values can be evaluated by a **Machine Learning algorithm** that recognizes (based on the training set) which values of voltage might belong to a positive case (having Influenza A) or to a negative case (not having Influenza A). In the literature, **Artificial Neural Networks** are used to recognize acute viral infection (Starodub, 2007). For our biosensor we would want to develop a similar system that takes conclusions of the voltage measured based on a training set. The model needs to be continuously improved to maximize accuracy.

We plan to manipulate the sample to 1) decrease the viscosity of the saliva and 2) stabilize the intended viral proteins in the sample. To achieve this, we would need to ship the Eppendorfs with different buffers. Thus, not adding any additional cognitive load to the end user of the tests. This would also require fluidics simulations to find the necessary buffer quantities to achieve the perfect fluid characteristics mainly to minimize the duration of the analysis in the gold surface. The cartridge is made of a $30\ \text{mm} \times\ 50\ \text{mm} \times\ 5\ \text{mm}$ glass card with a $5\ \text{mm} \times\ 30\ \text{mm} \times\ 2\ \text{mm}$ hole inside to guide the flow of saliva through an inlet to the functionalized gold surface.

We also want to reuse the cartridges by collecting the used cartridges from the pharmacies and taking them to a hydrogen peroxide disinfection and sterilization centre, this would reduce material costs, namely the gold chips and the glass card. The idealized biosensor and cartridge are presented in Appendix A – Figure A8 and Figure A9.

For the **immobilization process** we used a new technique that consists of creating a polydopamine-ethanolamine film on gold surfaces by chemical methods. We tried two different methods: one with polydopamine-ethanolamine and one with just polydopamine. To ensure that the film

was created on the gold surface and to study the differences between the two immobilization methods we used ellipsometry. After the films were created, we immobilized IgG antibodies on the surface and then immobilized again with anti-IgG antibodies. The ellipsometry data is shown in the graph bars in Appendix A - Figure A10. By analysing the data, we were able to calculate **the thickness of the layers**. Due to the ethanolamine properties, it was expected that the film created with both reagents was thicker than the film created with just polydopamine.

It is important to keep in mind that this was just **a proof-of-concept**. We only tested with the concentrations mentioned above and used IgG antibodies that were not of our interest, but still got relevant conclusions. In the future, we can test with **different concentrations** and **proteins and antibodies of interest to Influenza** to get more reliable results. Our focus in the future should be **finding the limit of detection** based on the different hemagglutinin and antibody concentrations tested in order to find **the lowest antibody concentration** our biosensor needs so that we can also **reduce the production costs**.

Originality

Identifying the **affinity reaction** between an immobilized antibody and a target antigen is fundamental for **disease control** (Felix, 2018). We need to guarantee a good biocompatibility between hemagglutinin and the surface. The ETA increases the thickness of the polymer layer and decreases the nonspecific interactions, increasing sensitivity and dispensing the addition of a blocking agent. We use Protein A to lower the concentration of antibody used, since it aligns the antibody on the surface increase number of binding sites. This leads to a reduction of the cost. Additionally, the **formation of a polymer layer is done in one step,** so it is simple and rapid to implement. The immobilization surface is very adaptable to any antibody. The ease of adapting this biosensor to any disease or test also **adds value to our product**. As for the cartridge technology, we idealized a **simpler mechanism** to collect saliva that does not involve microfluidics. The fluid will not be as controlled; however, we gain in **cost efficiency** and the test is **performed** by the user. We plan to collect the used cartridges aiming to **decontaminate and recycle the used material**.

"*The biosensor device idealized by the team from University of Lisbon, targeting the detection of Influenza A in saliva, relies on innovative approaches and also on previous scientific discoveries, as a result of an extensive literature review. The novelty behind the sensing interface, sampling process and optical detection enables the construction of a sensitive and cost-effective optical device, compared to other optical systems commercially available.*

One of the crucial steps to consider when developing a biosensor is the construction of the transducer matrix, including the efficient immobilization of the bioreceptor, in this case the Hemagglutinin/HA polyclonal antibody, and the ability to inhibit non-specific protein interactions. Commonly, there are a number of critical steps involved in biofunctionalization of gold surfaces. For instance, the deposition of an organic layer followed by a chemical activation of the pendent binding groups for the covalent attachment of proteins, which is performed in a subsequent step, and finally, the addition of blocking agents (e.g. albumin), to avoid non-specific interactions. Instead, LxUs team proposes a novel and disruptive strategy to prepare reproducible and low-cost, ready-to-use biosensor interface for hemagglutinin detection, that uses a hybrid polydopamine-ethanolamine film, not requiring additional chemical coupling reactions for biomolecule immobilization, or blocking steps throughout analyte detection.

Dopamine mimics the major aminoacids present in mussel's foot proteins and can be easily polymerized to polydopamine onto virtually any surface, including gold, under aerated and slightly basic conditions. Besides being biocompatible, polydopamine is highly adhesive and its quinone moieties can react with amine groups of proteins, providing a robust covalent linkage, without the need of extra coupling agents. The conjugation of ethanolamine, during dopamine chemical polymerization, ensures a good distribution of protein and will avoid non-specific adsorption during hemagglutinin detection, due to the presence of hydrophilic hydroxyl groups on the surface. To enhance the detection of the target analyte it is also proposed the use of Protein A to achieve a better orientation of the immobilized antibody. The Team is therefore proposing an innovative optical transducer interface to retrieve the main goal of SensUs competition 2021 - a saliva-based influenza Biosensor. Polydopamine is a very cheap and versatile material with many technological applications, although to my knowledge it is not yet a commercial solution in the biosensing field. The originality of the proposal does not end on the gold recognition interface but extends to the overall design of the optical device, including the simple method envisaged for saliva collection and straightforward analysis in the glass cartridge, without the requirement of tricky and expensive microfluidic and peristaltic systems.

I was very impressed with the overall commitment of the Team members to the project throughout all the meetings we had, including some preliminary laboratory assays to validate the proposed transducer interface. The urge to create a versatile, user-friendly and low-cost device was always on their minds and drove every discussion with scientists and market segments (pharmacists) to provide end-users with an efficient device. The final document reflects the quality of their work and the rigorous evaluation of proposal potential and associated risks."

Ana S. Viana,
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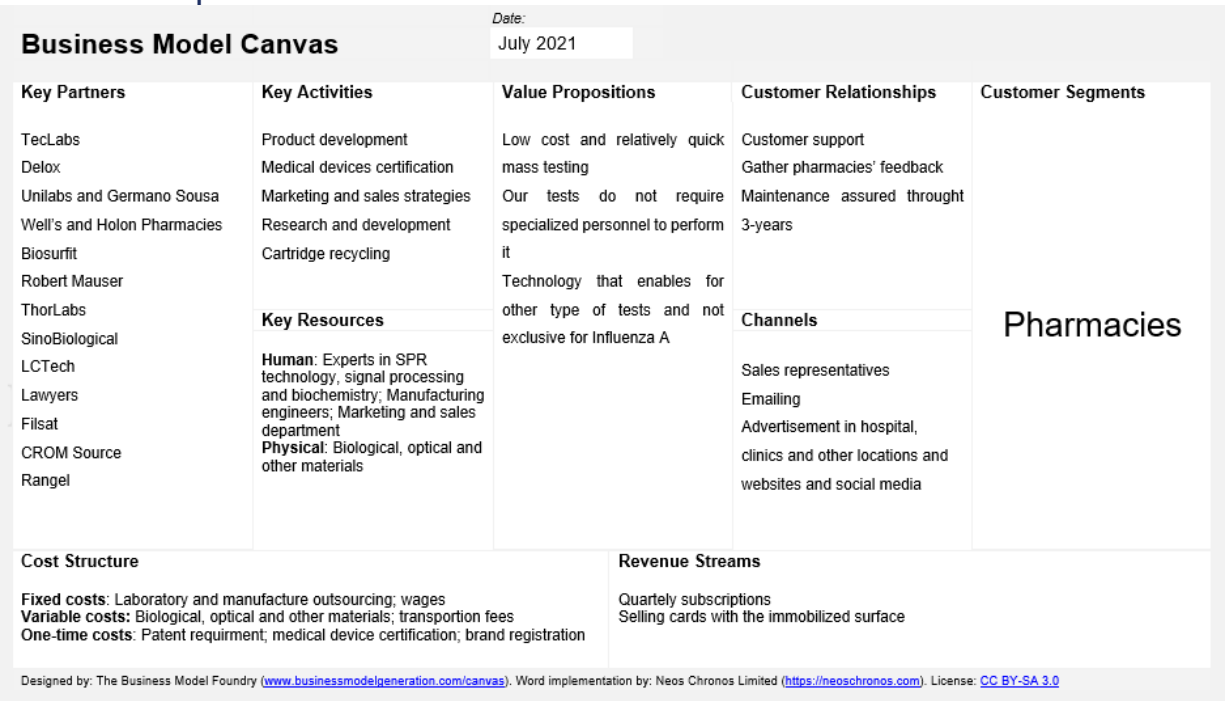
Catarina Moura, Team Captain

Rafael Cruz, Team Captain

Translation potential



Business Model Canvas

Date: July 2021

Key Partners	Key Activities	Value Propositions	Customer Relationships	Customer Segments
TecLabs Delox Unilabs and Germano Sousa Well's and Holon Pharmacies Biosurfit Robert Mauser ThorLabs SinoBiological LCTech Lawyers Filsat CROM Source Rangel	Product development Medical devices certification Marketing and sales strategies Research and development Cartridge recycling **Key Resources** **Human**: Experts in SPR technology, signal processing and biochemistry; Manufacturing engineers; Marketing and sales department **Physical**: Biological, optical and other materials	Low cost and relatively quick mass testing Our tests do not require specialized personnel to perform it Technology that enables for other type of tests and not exclusive for Influenza A	Customer support Gather pharmacies' feedback Maintenance assured throught 3-years **Channels** Sales representatives Emailing Advertisement in hospital, clinics and other locations and websites and social media	Pharmacies

Cost Structure	Revenue Streams
Fixed costs: Laboratory and manufacture outsourcing; wages **Variable costs:** Biological, optical and other materials; transportion fees **One-time costs**: Patent requirment; medical device certification; brand registration	Quartely subscriptions Selling cards with the immobilized surface

Figure 1 – Business Model Canvas designed for our biosensor.

Customer Segment

At first, we wanted to design a **point-of-care biosensor**, such as a thermometer. Thus, we ran a survey to understand people's needs and what they were willing to pay for such a device. The **range of prices** which had the biggest representation (36.3%) was **10-20€**. The results from the survey can be found in Appendix C, section C.1. This range of price could not perform with the technology we chose for the biosensor, SPR. Having in mind that with the pandemic, Portugal, and other European countries, began to have rapid testing in pharmacies, pharmacies have developed strategies, especially related with logistics, to handle testing. So, we thought the **pharmacies** would be the best target for our idealized biosensor.

Furthermore, we did **the validation for pharmacies in Portugal**, so it makes sense to promote our biosensor in the Portuguese market in a first approach. The business model needs to be re-considered when moving to other countries, given that different countries have different accesses and needs. The results of this survey helped us developing the business model presented and can be found in Appendix C, section C.2.

Value Proposition

Unlike the rapid COVID-19 tests that require a professional to collect the samples with a swab, **our tests would not require specialized personnel to perform it**, since the saliva sample is collected by the user with an Eppendorf. Through a meeting with a pharmacist, Gilberto Batista, we realized that the COVID-19 test is a burden for pharmacies, since they require a pharmacist to perform the test, preventing the realization of other functions. We plan to add value by providing **low cost** and **relatively quick mass testing**. This can be achieved by partnering with pharmacies by adding a **new revenue stream** to their already incumbent business. By providing extra income to pharmacies, the implementation of our product will be a net positive on the business and consequently ours, which will allow us to scale to as many pharmacies as possible. This will finally **unlock the economies of scale in SPR technology** and will allow it to thrive, being a highly versatile testing method. In a later stage of the adoption curve, and if our R&D makes it possible, **this testing does not have to be exclusive to Influenza A**, but it can be expanded to several other viruses and diseases, increasing the total addressable market of our product, while marginally increasing manufacturing costs. More information regarding validation to build this business model can be found in Appendix C, section C.2 and C.3.

Channels

Initial contact will be made directly reaching out to our key partners via **sales representatives**, with a very deep understanding of the fundamental concepts that make SPR a promising technology, to promote the biosensor not only in the big pharmacy chains but also local ones.

To reach the larger pool of independent pharmacies we devised a list of contact steps, starting with a wide-reaching method such as **emailing and, eventually, phone calling each pharmacy with a brief description of our business model** and **the possible cash-flow increases to their bottom-line**. In more densely populated zones, we can deploy our sales representatives, as they are a more effective vehicle of information and can answer any questions in real-time helping with engagement and consequently increasing interest in our product. Given that this is a product aimed at the general public, we can also deploy **advertisements** with the advantages of our tests to areas like hospitals, clinics, and other locations where the interest in our tests is higher than average.

Customer Relationship

The value proposition requires several activities that we have to do to appeal to our customer segment and enable the further improvement of our biosensor. Having this in mind we plan to have **customer support** not only to solve issues with the biosensor but also to provide the necessary cartridges to pharmacies. Our service and product need to be in accordance with the customer needs, and so we will have to **gather pharmacies' feedback every trimester** by a **telephone call**. Additionally, we want to provide **maintenance of the biosensor free of chargers** for the first 3 years of contract.

Business feasibility

Key Resources

For our business model to be successful, we need access to very **qualified engineers**, experts in SPR technology, signal processing, saliva handling and immobilization of surfaces and software. There will also be a very strong **demand for manufacturing engineers** to help to make the manufacturing very lean, minimizing capital expenditure, especially in the manufacturing of the sensing apparatus, seeing that will be the primary hurdle to mass adoption of this technology.

We must secure contracts with **material suppliers** for the biological material, optical equipment and all the electronics necessary to build our product. There will also be a very strong need for material suppliers of things such as plastic. Our goal is to bring as many processes in-house as possible, to maximize margins, reduce reliability on external factors and make the technological progress of our product as quick as possible. Finally, we need to approach several pharmacies, so we will need **a marketing and sales department specialized** in getting our biosensor across Portugal and eventually, other relevant geographies.

Key Activities

To reach a wide customer-base, our primary concern is to try to arrange **pilot-programs with major pharmacy chains and analysis centres as a proof-of-concept**. This is so that once our concept is proven successful and the business model around it is sustainable, it is very easy for these chains to implement our product across their locations, seeing that we have the ability to negotiate delivery contracts and cut on logistics costs and even reduce their costs by **taking advantage of their existing logistics network**.

We will also have product development to improve the signal processing algorithm and answer customer's needs and complaints. Given the earlier stage of our biosensor, **research and development is essential to increase the sensor's sensibility**, which is crucial to the reliability of our product. We want to understand if **a request for a patent is realistic** and submit documents to **a medical devices certification**. The cartridge recycling will also be fundamental for our business.

Afterwards, the marketing and sales department will need to develop **strategies** to bring the biosensor to Portuguese pharmacies and eventually expand to other pharmacies in Europe. For instance, if there is enough product output, we will partner with willing independent pharmacies to start building our own logistics network and starting to vertically integrate our products, consumables and in-house distribution. This can be achieved by **increasing the subscription prices for new consumers**, or by securing partner funding and **making the partnership completely free of charge** to the pharmacy for the first 12 months of a 24-month partnership.

Key Partners

Finally, we will need partnerships that enable our business model to work. First, we need to contact **TecLabs**, which is an Innovation Centre based in Lisbon, which will provide us **laboratory access through outsourcing**. To achieve our key activity of **pilot-programs** we must get in contact with **two major analysis centres**, **Unilabs** and **Germano Sousa**, and two major pharmacy chains, **Wells** and **Holon**, all based in Portugal. Additionally, **Biosurfit** is a Portuguese company that has **worked with SPR** before and **sells rapid tests with saliva for COVID-19**. Thus, their expertise will be essentially to further develop our business model and biosensor.

Regarding cartridge recycling, we need to create a partnership with **Delox**, which is a Start-up that would handle the decontamination of each card so it can be used more than once. Another important partnership is with **companies that supply electronic, optical and biological materials** to build the biosensor and the immobilized surface. For that we have chosen **Robert Mauser**, **ThorLabs** and **SinoBiological** and **LCTech**, respectively. We will need to contract **lawyers** for legal issues and eventually, if patent preparation is done, to submit a patent.

We found that **Filsat** would be a good fit to assist in **sales of medical devices** and **CROM Source** as our **medical device consultor**. We will need to find a good partner that can manufacture our biosensor. However, this partnership will be carried out after the results of the pilot versions. Finally, for **transportation**, we want to make a partnership with **Rangel**, since they hold logistics for the Pharmaceutical Industry.

Financial viability

Revenue Streams

Our business model relies on the recurring revenue of each pharmacy in the form of a **quarterly subscription**. This subscription can be tailored to fit the needs of each individual pharmacy, **putting their experience first**, and most importantly, **assisting in increasing revenue for both parties involved**. As the business grows, it is important to maintain the granularity in our subscription model, meaning that all the financials, order volumes and logistics need to be handled on a case-by-case basis, offloading as much of the back-end work to our business, to reduce the amount of man hours necessary to maintain the testing apparatus up and running, maximizing the profit per test. This is extremely important because **the less the pharmacy has to do to support our tests, the more likely it is for them to buy our product and make money from it**, ultimately increasing product volume and making it accessible to a wider range of pharmacies across a wider demographic.

More specifically, this **subscription model** includes a **fixed cost** for the rental of the equipment, (that includes all servicing and calibration costs), and a **cost per test**, depending on the amount of tests agreed in the contract. In the trial stage of our deployment and depending on the funding we can collect to bring our product to market, this **subscription fee can be tailored to maximize reach** (negative gross margin, or 0 gross margin), and after the early phase of our deployment, we can manage the subscription fees to become profitable, especially because as the business grows, we get more operating leverage, that increases gross margins. More information regarding revenue streams can be found in Appendix D.

Cost Structure

Our **fixed**, and therefore **predictable costs** will be mainly related to logistics, lab rental and other manufacturing costs, such wages and leasing costs. The **variable costs** will be the **materials**, which will depend on how many cartridges and biosensors we are producing and will vary with economies of scale and the transportation fee. We want to arrange a contract with a variable fee that is dependent on the number of packages we send. The **one-time costs** will be the **submission for a possible patent and brand registration**, which will require lawyers dedicated to patents and the bureaucracy, as well as **medical device certification**. More information regarding cost structure can be found in Appendix D.

Team and support

Team Members (by alphabetical order):

- **Catarina Moura**: Catarina is studying Biomedical Engineering. She was one of the Team Captains and was part of the Biosensors, Social and Business Team. She was responsible for maintaining the communication between the SensUs organization and the whole team, as well as to keep track of deadlines and medal progress.
- **Inês André:** Inês is studying Biomedical Engineering. She is part of the Cartridge Team and participated actively in the Entrepreneurship Sessions. Since she is a very talkative person, she has attended an event from another team and has joined in the SensUs Community Video.
- **Filipa Neves:** Filipa is studying Biomedical Engineering. She was part of the Biosensors and Business Team. She was also the person chosen to be the team promoting person (TPP).
- **Madalena Pereira:** Madalena is studying Biomedical Engineering. She was part of the Cartridge team and helped idealize the idea conception process, as well as the management of the saliva sample.
- **Mariana Almeida:** Mariana is studying Advertising & Marketing. She was part of the Business Team and helped develop the brand, manage and create content for the team's social media, and gave helpful insights in the areas that were not the expertise of the majority of the team.
- **Mariana Oliveira:** Mariana is studying Biochemistry and was part of the Biochemistry team. She collaborated in the innovation of the immobilization method.
- **Marta Bento:** Marta is studying Biochemistry and was part of the Biochemistry and Social Team. She collaborated in the innovation of the immobilization method.
- **Nuno Taborda:** Nuno is studying Physics Engineering. He was part of the Biosensor Team. He collaborated in the development of the detection method and other hardware tasks.
- **Rafael Cruz:** Rafael is studying Biomedical Engineering. He was one of the Team Captains and was part of the Cartridge Team. He was a facilitator in the idea conception process and then to connect the ideas between each development team.
- **Rodrigo Cordeiro:** Rodrigo is studying Biochemistry. He was part of the Biochemistry team. He collaborated in the innovation of the immobilization method.

Our journey would not be possible without the support of professors, medical professionals, pharmaceuticals and members of last year's LxUs Team. Firstly, we want to thank to **Prof. Hugo Ferreira** and **Prof. Ana Viana** for their support. Prof. Hugo helped us with the concept of the biosensor and business model. Prof. Ana played a central role in our journey, given that she works with SPR in her lab and had recently published an article with a new immobilization technique, in which our concept was based.

We also want to thank **Prof. João Coelho**, **Prof. Luís Coelho**, **Prof. Annelies Bobelyn**, **Prof. Margarida Carvalho**, **Prof. Paulo Paixão** and **Prof. Ana Prata** for helping us in different phases of our project. It is important to mention that Prof. Ana Viana and Prof. João Coelho enabled our access to the lab when possible. During our market research, we had the chance to meet the **pharmaceutical Gilberto Batista** that offered his insight and time on our business model, particularly in the value proposition.

During these months we also took advice from our colleagues from **last year's team**, especially regarding budget problems and team management. Finally, we want to thank **Marta Oliveira** and **Rui Guilhôto** who made part of this year's team at the beginning and helped us especially with literature review. The project presented here is the result of all the meetings and insights we have collected through these months.

Final Remarks

We believe that SPR technology is promising for rapid testing with significant sensibility. Thus, our first goal would be to develop an **affordable detection apparatus with SPR** that would detect hemagglutinin from saliva samples. Unfortunately, we had no budget for this year's team, and even though we looked for partners to support us, we had no luck. We believe that the ongoing uncertain pandemic context diverged our potential funding partners priorities away. So, it was not possible to run any kind of tests in a prototype or even develop a prototype. This, of course, led to lack of information in the Technological Feasibility chapter, only possible with experimentation and material, and estimation errors in the Financial Plan, that we tried to tackle the best we could.

Two of the biggest challenges of this project are the **reduction of the SPR price** in the market and **the collection of saliva** we idealized. Apparatus with SPR technology have been in the market for several years. Biacore, which we believe to be the one of the biggest companies in SPR, released **its first SPR instrument in 1990**. We would have years of knowledge to keep up with and inevitably less budget. Thus, **partnering up with Biosurfit**, which has experience in both SPR and testing with saliva, would be a fundamental advantage for us. As for the handling of saliva, we idealized it based on the papers and information we gathered in conferences organised by Salimetrics. However, we recognize that **it would be of value to test this method** in a laboratory to understand if a **reliable alternative** for the microfluidic systems in the market was found and **further improve it** with experimental data. Since saliva does not behave exactly like water, given that its viscosity can be about 5-30% higher than water.

The choice of our immobilizing surface also came from the fact that saliva consists of a mix of many components, and we only want to detect hemagglutinin. Consequently, we want to design **low-cost immobilization surfaces** while maintaining its **selectivity**. This poses a compromise because we cannot have both at the same time, we must try to maximize what is more important to our biosensor. Even though we had the chance to go to Prof. Ana Viana's lab once and performed tests with gold surfaces, it was not enough to take conclusions and improve our immobilization surface for Influenza A and perhaps other type of bindings between antibody and antigen to further validate our business model.

This project was fundamental to recognize the **importance of having a budget**. Being informed of this was discouraging enough, but once we tried to create partnerships and had no success, it was even more demotivating. Inevitably, it took a toll on the team's spirit. This kind of projects do not rely on theory solely, especially to further improve our ideas and understand their feasibility. However, we made the most of the competition by **participating in the events organized by SensUs** (Entrepreneurship Sessions, Partner Sessions and Feedback Moments) which were enriching for our project, and we also had the chance to **meet other team members** from different teams and backgrounds through the events they organized. Therefore, all the work presented in this report is the result of our resilience and capacity to make the best out of unfortunate situations.

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Appendices
Appendix A: Figures

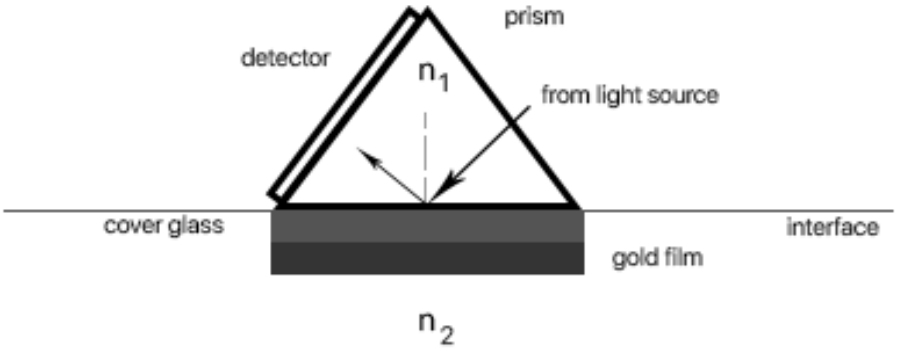

Figure A1 - Representation of the Surface Plasmon Resonance (SPR) concept (Tang, 2010).

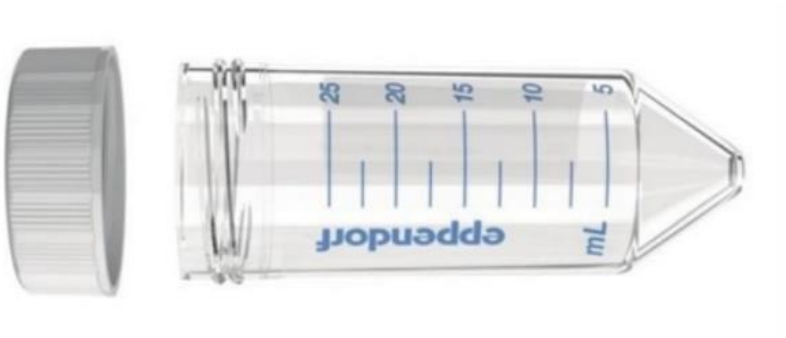

Figure A2 - Eppendorf used for cartridge (FisherScientific, 2021, July 25).

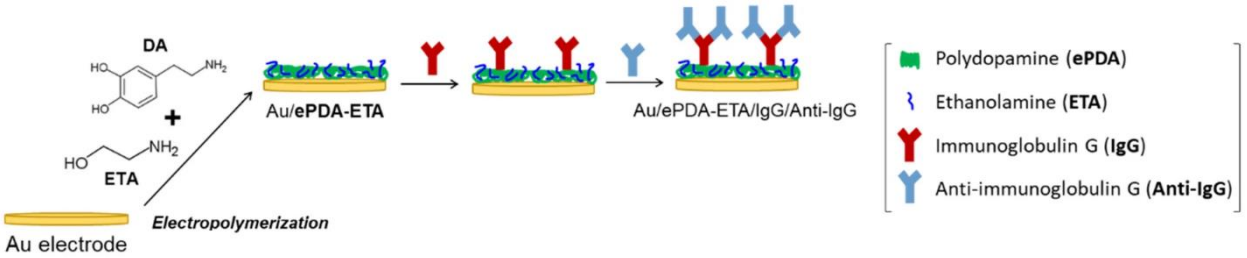

Figure A3 - Immobilization surface polydopamine-ethanolamine where Immunoglobulin G is used as example for the antibody. DA stands for Dopamine hydrochloride (Almeida, L. C., 2021).

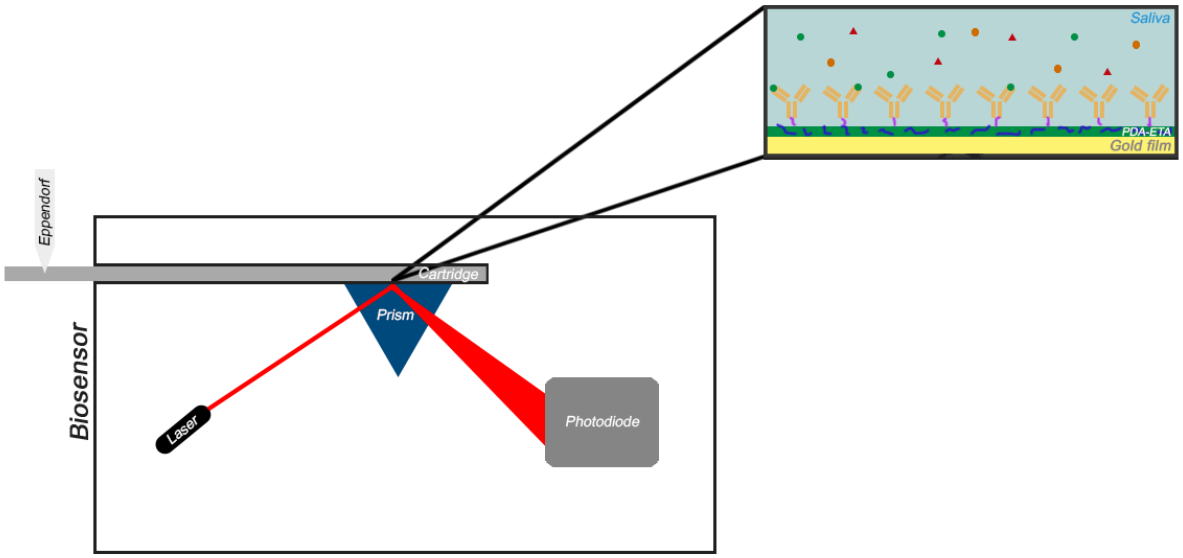

Figure A4 – Schematic of the idealized biosensor with SPR instrument and immobilization surface inside the cartridge. The green dots represent hemagglutinin, the other dots represent other elements of the saliva and in purple is the Protein A. The binding antibody-antigen is represented as well.

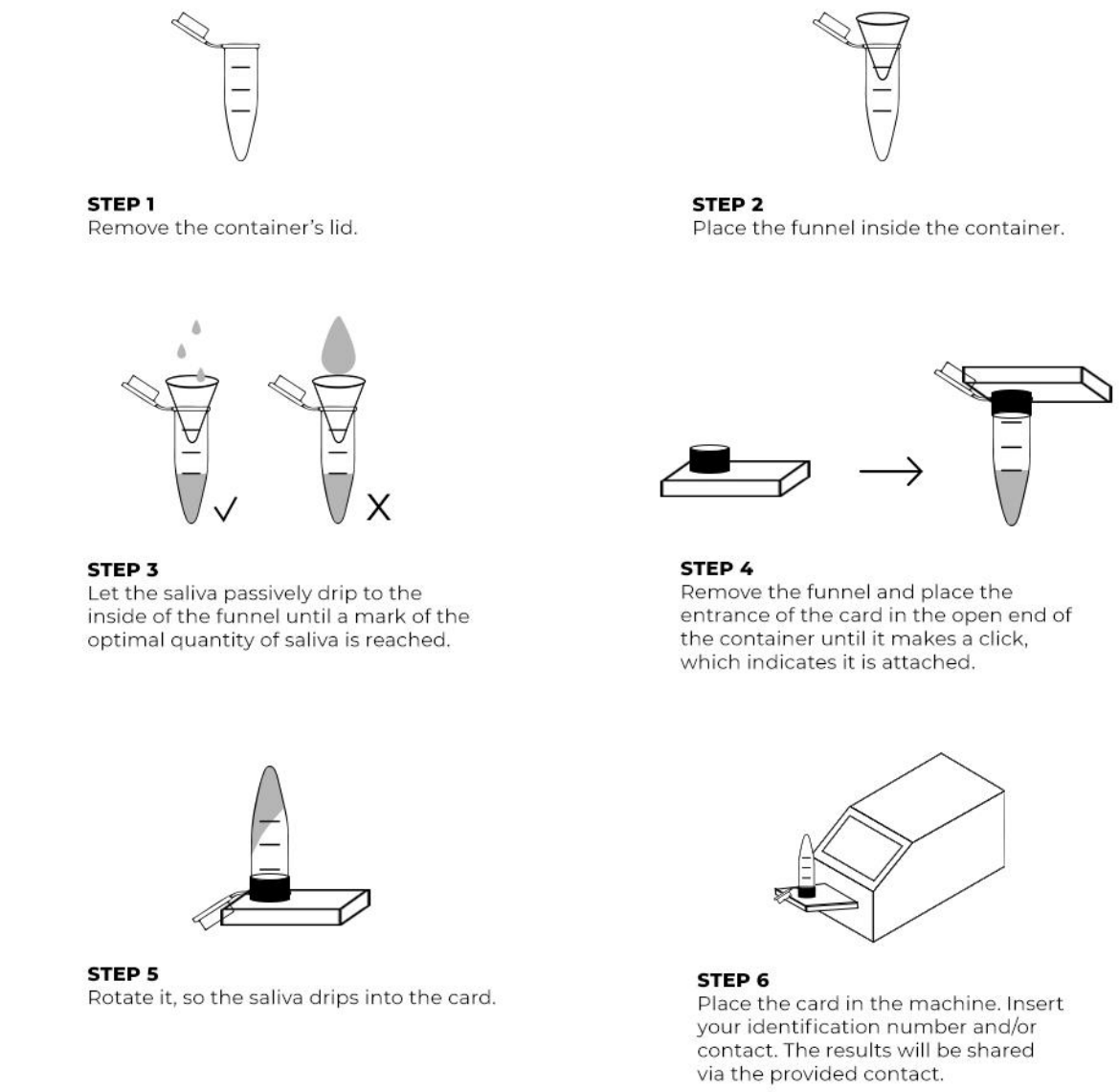

Figure A5 – Instructions placed in the biosensor to inform the user on how to perform the test.

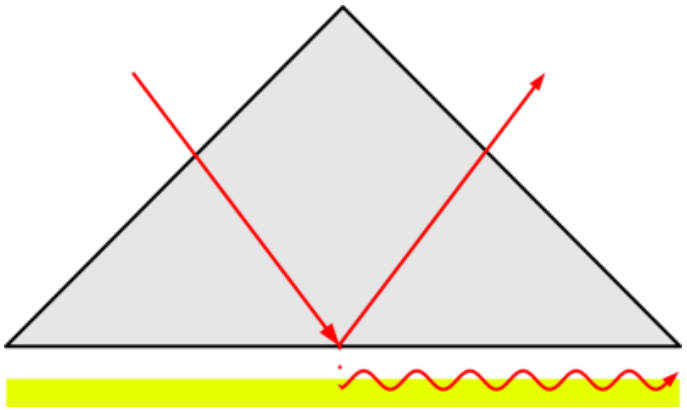

Figure A6 – SPR with prims in Otto arrangement (Wikipedia, 2021, July 25).

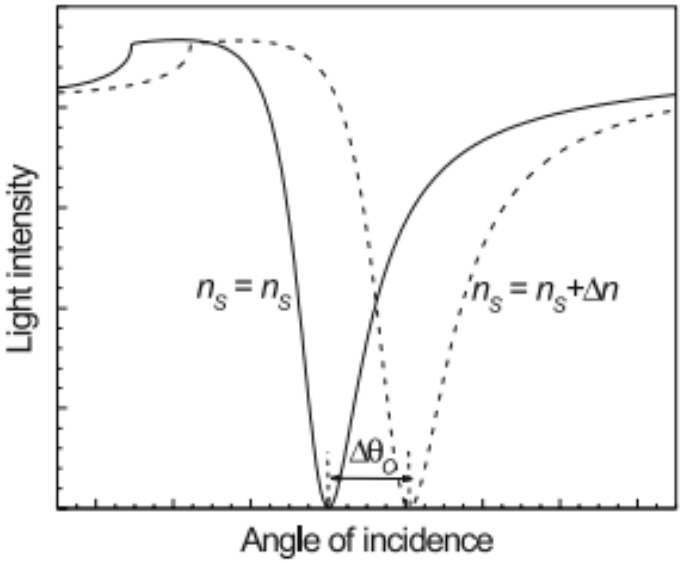

Figure A7 – Intensity of light wave as a function of angle of incidence (Homola, J., & Piliarik, M., 2006).

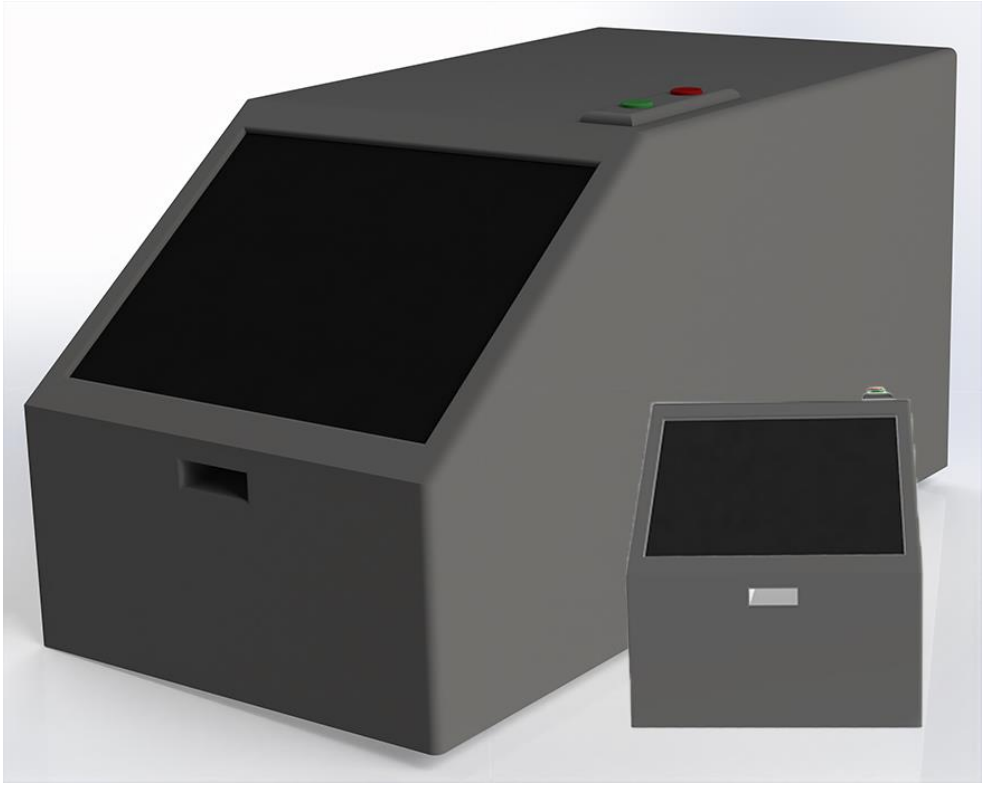

Figure A8 – External view of biosensor placed in the pharmacies.

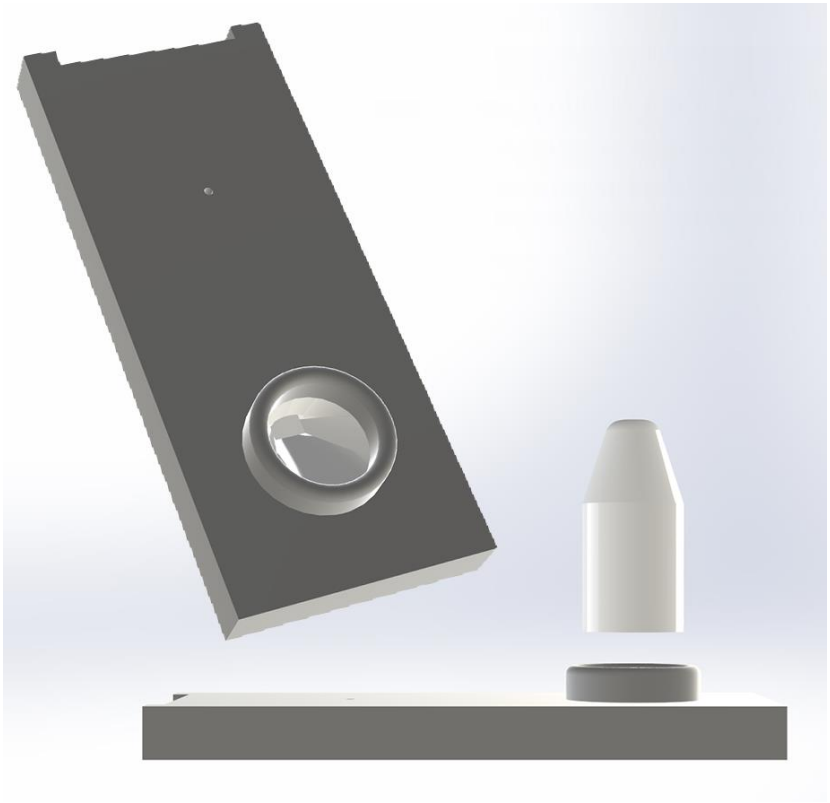

Figure A9 – External view of cartridge with Eppendorf.

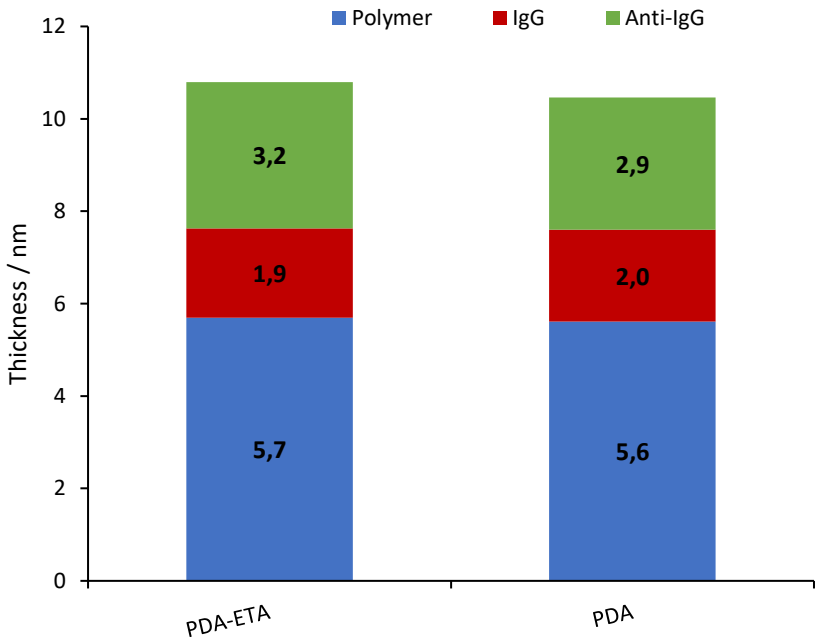

Figure A10 - Graphic representation of the thickness, in nm, of the PDA-ETA (left) and PDA (right) layers, respectively.

Appendix B: Impact of technological progress on prices

One factor that drives long term economic growth is **technological change** (Solow, 1956). The **technological progress** can be divided into three phases: **invention, innovation, and diffusion** (Neisser, 1999). The invention phase represents the creation of the idea. In the innovation phase the idea is applied to solve real world problems and finally, the diffusion phase symbolises the market entry and can be further divided into several substages: niche market commercialization, pervasive diffusion, saturation and senesce (Grübler, 1999).

The **technology's life cycle** is defined by **cost level and market share** (Wagner, 2014). Naturally, technology costs are high during the invention and innovation phases, but **these costs start to decrease** during the diffusion phase, mainly due to **the economies of scale and learning effects**. The knowledge transfer indirectly leads to lower costs.

A company might not choose to move prices along with costs. In **a niche market stage**, the company can set **the prices lower than costs** with the expectation of capturing a bigger market share. The fact that prices do not have to decrease along with cost allows competitors with elevated production costs than the market leader to enter the market. By **increasing the competitiveness**, the market leader is compelled to make changes, including decreasing prices. This is called the shakeout phase. With competitors in play, the price is forced to follow cost. As for SPR technology, we believe that it is an extremely adaptable diagnostics tool since it only depends on an interaction between analytes. Only a few companies build and sell SPR instruments, where the market leader is Biacore. Usually, higher prices are charged in niche markets and for now, the market surrounding SPR is a niche. Policy measures can be created in order to encourage further technology deployment. The sources of cost reduction are described in the literature (Solow, 1956) and presented in Figure B1.

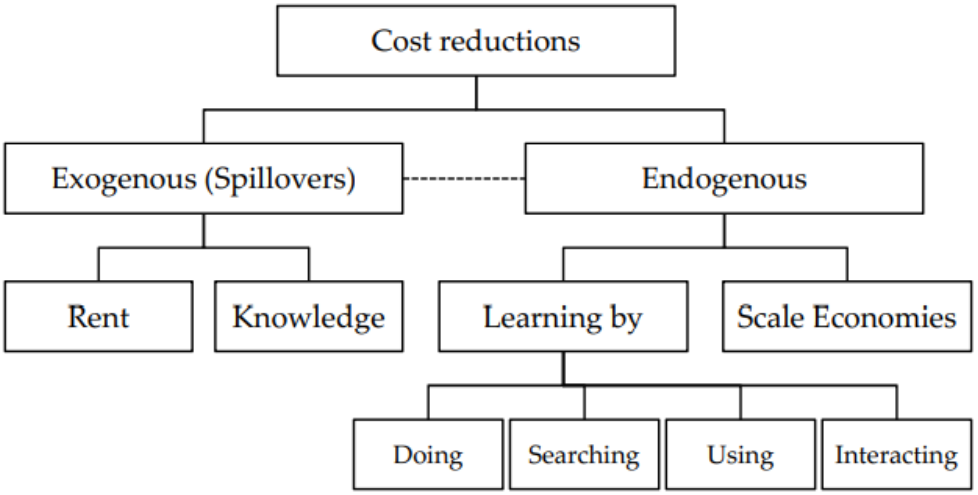

Figure B1 - Sources of cost reduction (Solow, 1956).

The **exogenous sources of cost reduction** result from transfer of economic benefits and/or knowledge. For instance, the reduction of material costs. The **endogenous sources** result from the learning effect and economy of scale. The accumulation of knowledge can result from several processes of learning: by doing (increased operational efficiency and effectiveness), by researching (also referred as Research and Development - R&D), by using (from user feedback) and by interacting (transfer knowledge within different groups).

The **relevance of SPR in the diagnostic market needs to be recognized** so there is more investment in this technology, which naturally drives a reduction of prices. This recognition might arise from an event such as a global pandemic. For instance, we can think of mRNA vaccine technology that was studied in the literature for years and only was introduced in the market due to the COVID-19 pandemic and the use of saliva for the diagnosis of respiratory viruses, that also started to be more investigated due to the SARS-CoV-2 virus.

Appendix C: Survey Results

C.1 – First Survey, November 2020

The aim of this survey was to i) understand the importance that people living in Portugal give to the flu, ii) understand whether people were afraid of going to the hospital because of the pandemic and iii) see what type of equipment people would prefer to test for the flu and the price range they were willing to pay. We had a total of **1080 answers**, 76.8% of them being women and 23.1% being men. The **survey was shared** across **social media** such as Facebook, Instagram and WhatsApp. The age groups are represented in Figure C1.

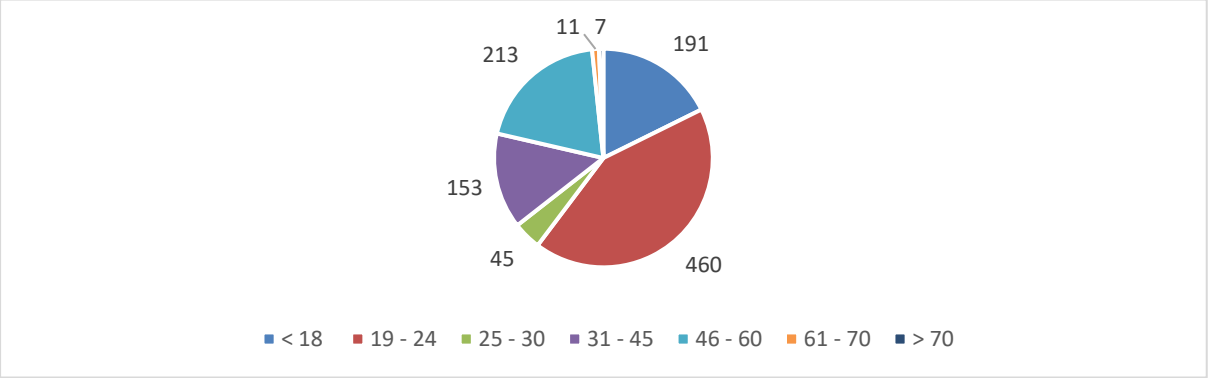

Figure C1 - Graph with the age groups for the first survey with 1080 answers.

We divided the survey in **two time frames**: before the pandemic and during the pandemic. Firstly, focusing on before the pandemic, we tried to understand what **people used to do when they had flu symptoms**, if they **found the fly worrying** and if they **were vaccinated against Influenza A**. The results are in Figure C2, C3 and C4.

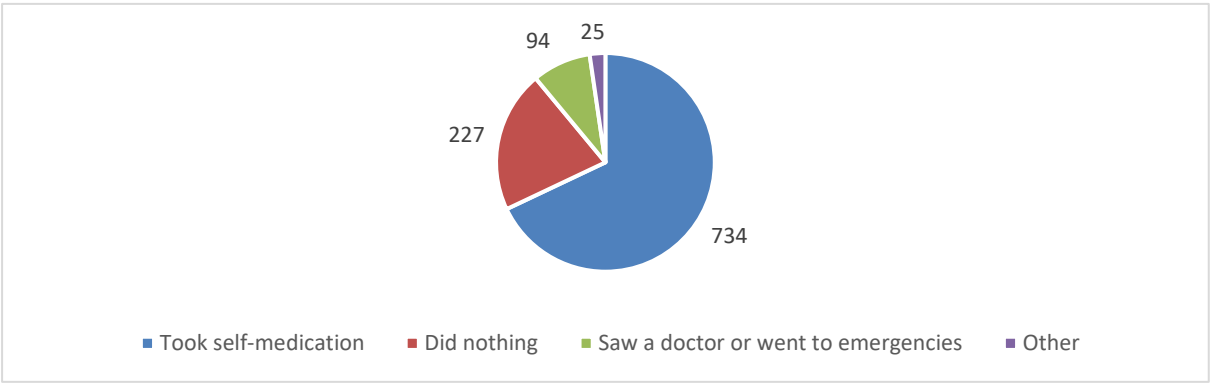

Figure C2 - Graph with the common approaches on dealing with the flu before the pandemic.

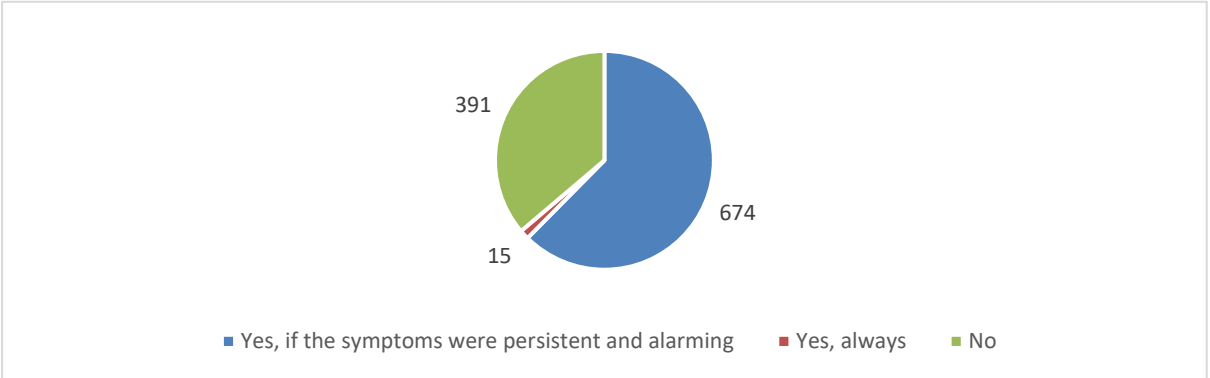

Figure C3 - Graph with the answers for “Did you find the flu worrying?”.

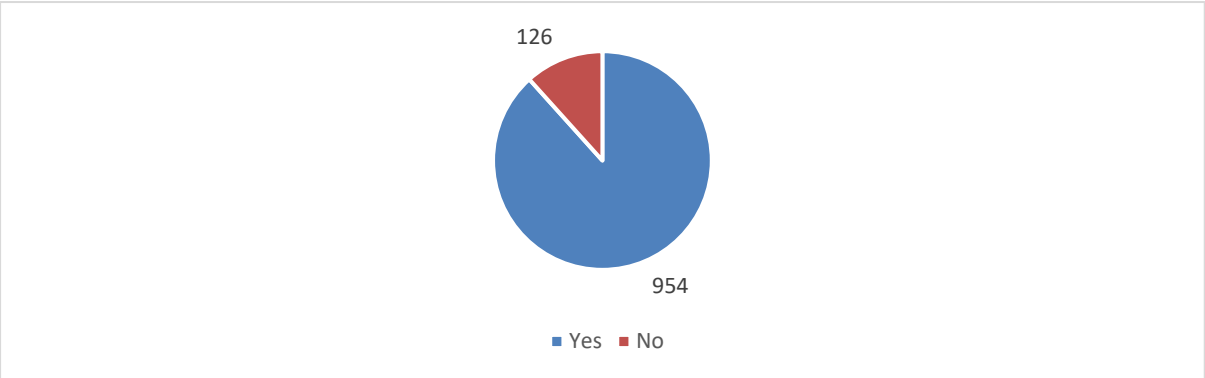

Figure C4 - Graph with the answers for “Did you receive the vaccine against the flu yearly?”.

Secondly, focusing on during the pandemic, we tried to understand if **people avoided going to the hospital** or to see a doctor due to the pandemic. About 74% did not feel afraid and 20% did but went anyway. The vast majority of the surveyed (73.4%) **became more worried with the flu** due to the pandemic.

The last section of the survey was about a **hypothetical device to detect the flu**. Most of the surveyed, 95.4%, were receptive with the existence of this type of device. The results for the **location of this equipment** are in Figure C5. The answers for the price range for this device are in Figure C6. Finally, 92.9% of the surveyed confirmed that they would trust this type of device.

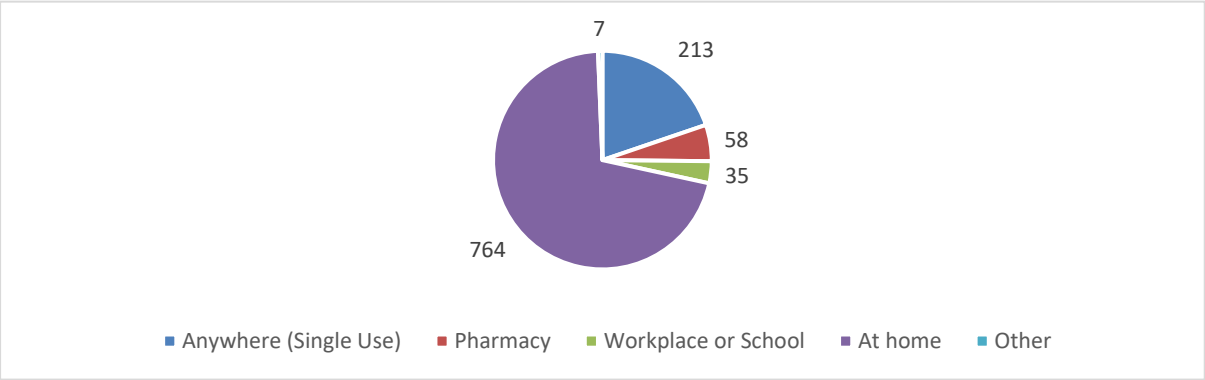

Figure C5 - Graph with the location of equipment outside of hospitals.

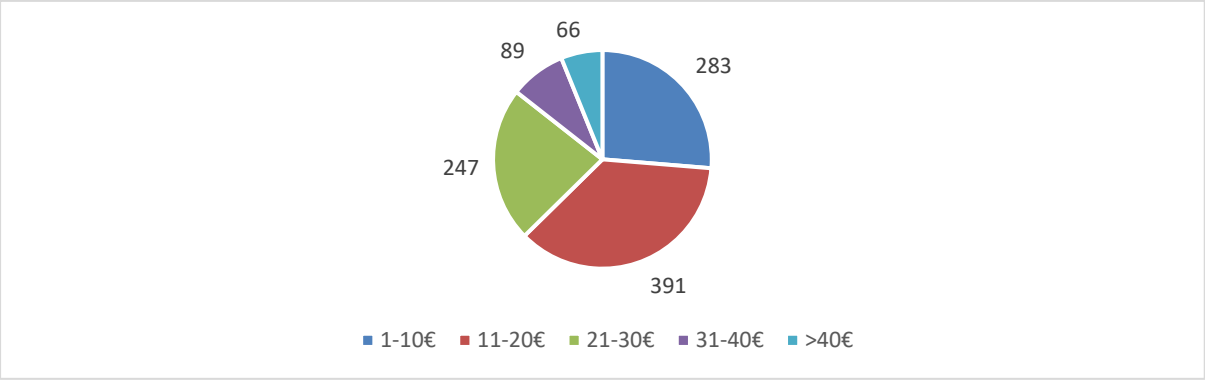

Figure C6 - Graph with the price ranges the surveyed were willing to pay for a device that could detect the flu.

This survey helped us to understand the **relevance of Influenza A to the general public** before and after the pandemic, as well as a **possible interest in a possible biosensor for testing**. Since we first release this survey, our idealized biosensor changed a lot, however we considered that this survey was a huge starting point for brainstorming.

C.2 – Pharmacies Survey, May 2021

The aim of this survey was to i) know how many COVID-19 rapid tests the Portuguese pharmacies do on average per week and ii) understand whether Portuguese pharmacies find Influenza testing relevant and if they were willing to do this type of testing. We had a total of **69 answers** across 17 of 23 districts in Portugal, including archipelagos - Azores and Madeira. The density of responses according to districts is represented in Figure C7.

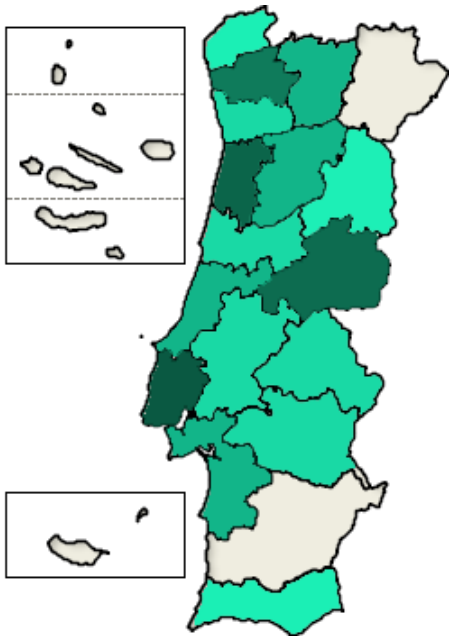

Figure C7 - Density of pharmacies responses to the survey across Portugal, including the archipelagos. The intensity of colour represents higher density of answers.

The survey was **shared across social media** such as Facebook groups related to pharmacies, Pharmacies' Facebook page, LinkedIn, and Email. The pharmacies' Emails were retrieved from Farmácias Portuguesas website. We sent **1035 emails** and messages through Facebook, from this 26 Emails were rejected. The distribution across districts of the emails and messages sent is presented in the graph in Figure C8.

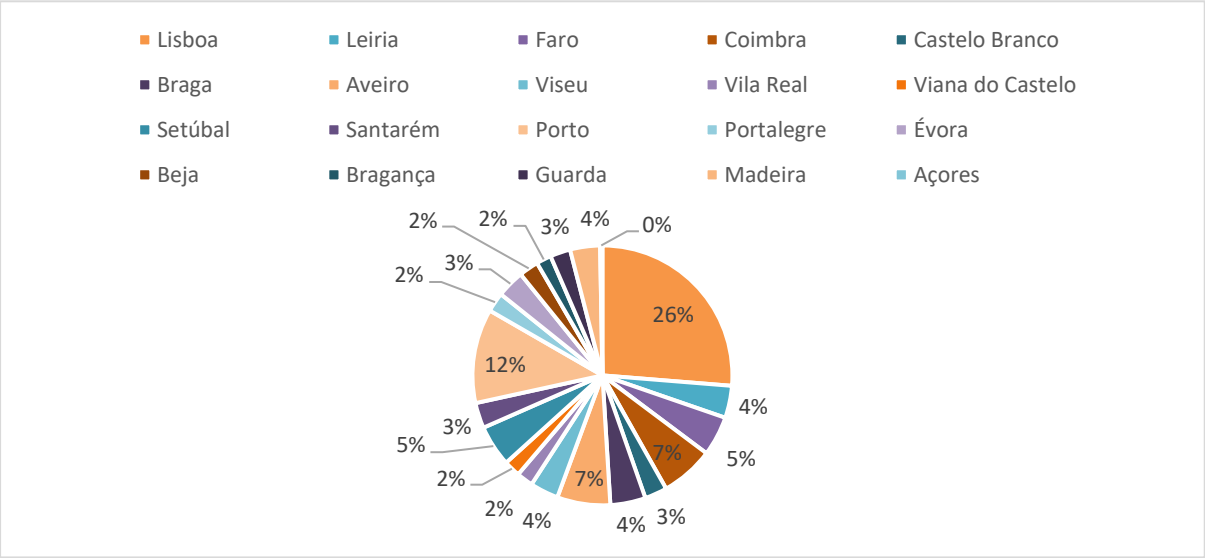

Figure C8 – Distribution of the locations of the pharmacies we contacted through Email, during May and July 2021.

Firstly, we wanted to know the function of the person answering to our survey. Our aim was to reach **as many people in leading positions as possible** since these peoples would be responsible to sign the contract to have our biosensor in their pharmacies. In total **25 people** (about 36% of the surveyed) were in leading positions (manager, owner and technical director) – the functions are represented in Figure C9.

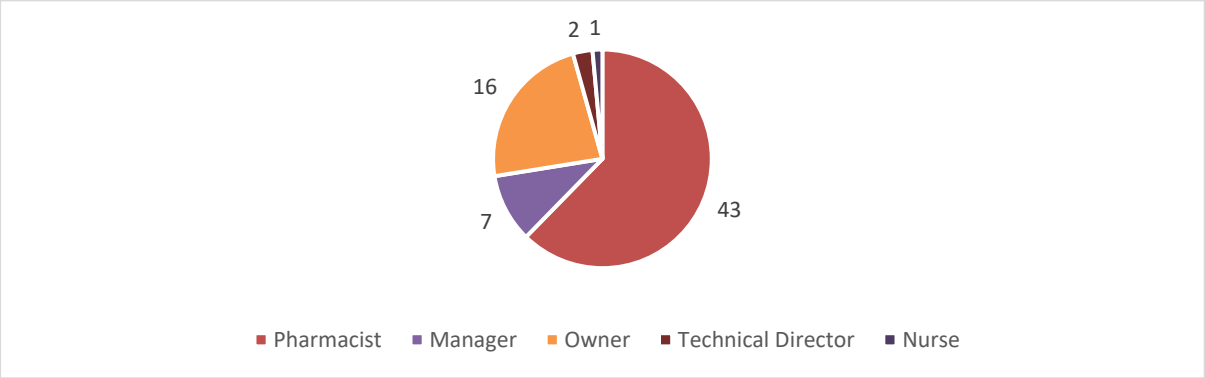

Figure C9 – Function of the person who works in the pharmacy.

Secondly, we asked how many rapid tests for COVID-19 these pharmacies perform in a week on average. About 46% of them, **perform only 10 to 40 tests weekly** – the rest of the results are presented in Figure C10. The **vast majority** (83% of the surveyed) **agreed with the existence of an option for rapid Influenza testing in pharmacies**. We obtained a higher percentage (of 84%) when only considering people in leading positions. However, the people who did not agree presented several reasons. These reasons presented a challenge because they bring up strong points that only led to the enrichment of the value proposition and the overall business model presented in the Translation Potential. Here we present some of the most relevant answers:

- "Impractical in terms of logistics."
- "Given the equipment presented, we might not have space in our pharmacy for it."
- "With the use of mask, the spread of Influenza has reduced drastically and besides that the disease is less debilitating, since vaccination prevents reaching critical stages."
- "Influenza has a trivalent vaccine and screening cannot be this spread, because they might create fears and a false sense of security."
- "The general population does not understand the difference between the flu and a cold. They take flu symptoms as a little cold and are not worried about getting tested."
- "The influenza test does not ensure the non-existence of other virus. The influenza virus is the one that less worries the population."

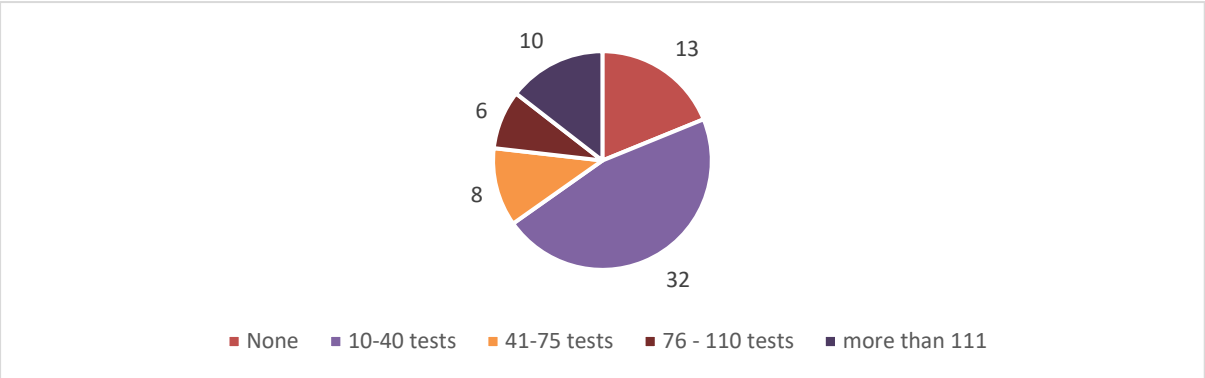

Figure C10 – Average of rapid tests for COVID-19 performed by the pharmacies weekly.

This survey was fundamental to understand whether pharmacies had interest in a biosensor for Influenza A testing, even though most surveyed were interested, the ones that were not raised very important points that we needed to consider. The "voice of the customer" is essential to determine the requirements of the equipment.

C.3 – Customer experience with Rapid Testing for COVID-19 in Pharmacies, July 2021

The aim of this survey was to i) understand how much people were willing to pay for a rapid test (in case for COVID-19) or how much they paid for one, ii) understand if they were satisfied with service provided by the pharmacies in testing and iii) know if more purchases in the pharmacies were made beside the rapid test. We had a total of **31 answers** during the month of July. The **survey was shared** across **social media** such as Facebook, Instagram and WhatsApp.

The Portuguese government has taken measures to encourage mass testing to mitigate the propagation of the SARS-CoV-2. One of these measures is the **subsidizing of COVID-19 rapid tests** in more critical areas. The majority of the surveyed (77.4%) had their test co-funded. For the people who had not their test co-funded (19.4%), the range of prices they paid are presented in the Figure C11. As for people who had their test co-funded, the range of prices they were willing to pay are represented in the graph of Figure C12.

We asked the surveyed to **classify the service provided** from 1 to 5, being 1 not satisfied at all and 5 very satisfied. About 42% of the surveyed were very satisfied with the service of the pharmacy in testing. Only one person was not satisfied at all. The rest of the surveyed classified the service with 3 and 4. The **biggest disadvantages** in getting test in pharmacies are the **need for booking the test** (32%), the **number of people in the pharmacy** (12.9%), the **waiting time** (9.7%), the **commute to the pharmacy** (9.7%), the **price** (9.7%) and the **test sensibility** (3%). About **23% of the people did not find disadvantages**.

We were surprised that most people (61.3%) did not find discomfort in an invasive test. When **asked if they purchased other products in the pharmacy besides the test**, 96.8% told us they did not. Initially, we had as a value proposition for pharmacies the possibility of people acquiring more products besides the test while waiting for the result, resulting in an extra revenue stream for pharmacies. However, **this survey served as an evidence to discard this possibility**. We believe that this was one of the most important survey for us since it demystified many pre-conceived ideas we had when first building the business model.

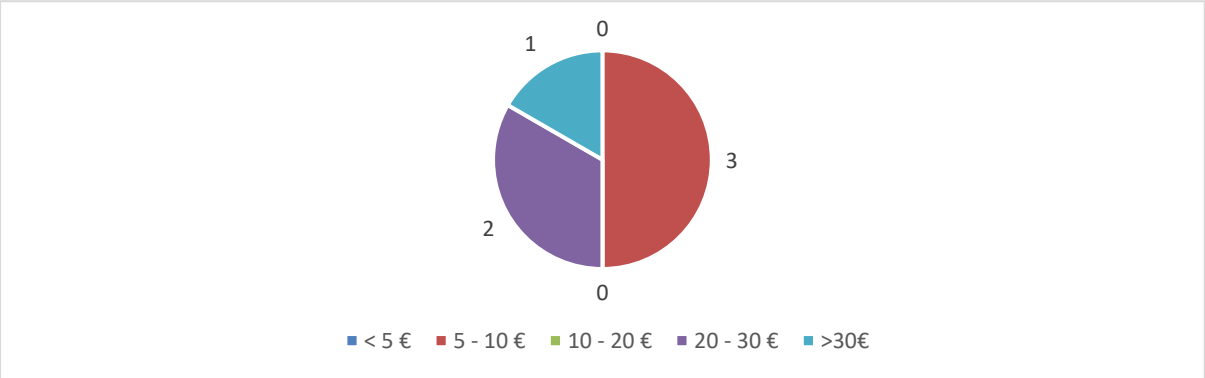

Figure C11 – Price range of a rapid COVID test in Portugal.

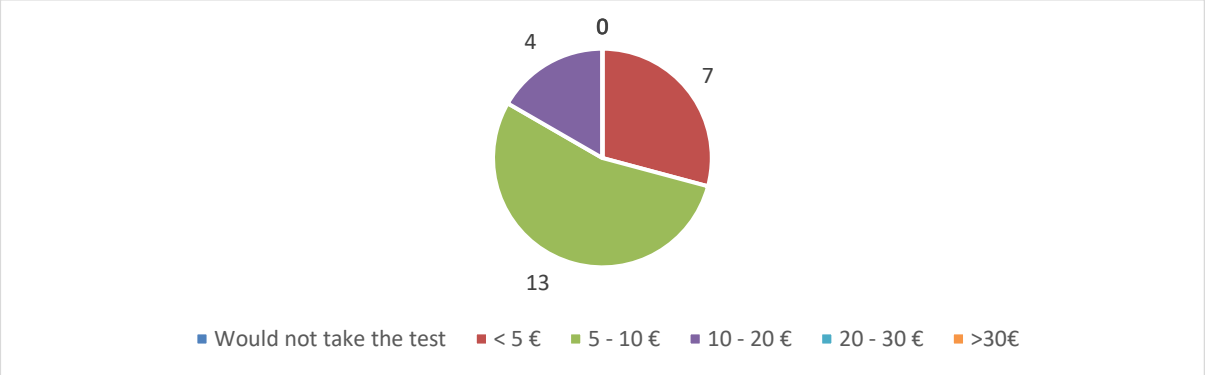

Figure C12 – Price range people were willing to pay for a rapid COVID test.

Appendix D: Financial Plan

D.1 – Rev-Cost Assumptions

The financial plan was projected for the first year of our company using the Financial Plan Template provided by SensUs Organization. For **human resources**, our company will need Biomedical (with expertise in optics and/or signal processing), Physics (with expertise in optics and/or signal processing) and Chemical (with expertise in immobilization of surfaces and SPR) Engineers, as well as App Developer, Product Marketing Manager and Sales Manager. The average gross monthly salary was retrieved from Paylab, Payscale and salaryexplorer. The estimation of the net salaries was obtained with the net salary simulator 2021 for Portugal. For the simulation, we considered that the employee lives in Portugal mainland, is unmarried and has 0 dependents on them. We considered a daily meal allowance of 7.63€ in a meal card (considering Ticket Services) for 22 days. Of course, this estimation has a greater error since not all employees will be in this condition.

Table D.I – Human Resources necessary for our project and their respective average gross monthly salary.

Function	Average gross monthly salary (in €)	Average net monthly salary (in €)
Biomedical Engineer	2,310	1,623.84
Physics Engineer	2,000	1,505.86
Chemical Engineer	1,278	1,110.28
App Developer	1,295	1,122.41
Product Marketing Manager	1,293	1,121.63
Sales Manager/Representative	1,219	1,079.77

For **the first 6 months of our company**, we are not considering hiring employees for Product Marketing Manager and Sales Manager, since we will be developing our product in terms of research with the engineers and app developer. During these months, we will need two biomedical engineers, two physics engineers, four chemical engineers and 3 app developers. Our focus is mainly with chemical engineers since our immobilization surface is the source of innovation and deserves the most attention. The biomedical and physics will be focused with the SPR technology, and thus ways of decreasing its cost, and signal processing.

As mentioned in the **Revenue Stream** in the Business Model (in the Translation Potential Chapter), we will make our profit from **the sales of cards/tests and from the subscription fee**. We may consider selling our biosensor to Universities and Analysis Centres once our biosensor is well enough developed. The **prices set for the cards/tests** is 25€/unit for a direct cost of 25€ to manufacture, the **subscription fee is 500€/month** for a direct cost of 0 € and **the biosensor 5,000€** for a direct cost of 6,000€. **These prices might be over or underestimated**, since we had no funding for our project, we could not correctly estimate the costs to set the right price. However, the direct cost per unit is higher than the unit price, since we want to **enter the market as a strong competitor** to the market leaders, as it was previously discussed in Appendix B. For now, we assume 0% for both fixed remunerations and index and accumulated salary increase in fixed costs for Human Resources.

The services rendered by third parties will be **Logistics and Delivery** that we estimate to cost about **700€/month**. It is worth mentioning that this merely an estimation, since the transportation company chosen by us, Rangel, has not a price table for these kinds of services and requires a budget request.

The **Infrastructure and Operational costs** will be placed on **laboratory outsourcing, space for self-storage, meeting room rental and travels to pharmacies** (for sales and/or technical support). All the prices include VAT. The **laboratory outsourcing** will be in TecLabs (located in the Faculty of Sciences of University of Lisbon) which will cost about **216€/month**. We estimate that the **manufacturing running costs** will be around **10,000€/month**. Having an office is not one infrastructure and operational cost for us, though it will be important to hold at least 2 presential meetings per month and for that we **rent a meeting room** at Spaces, which is located at Marquês de Pombal (centre of Lisbon) for a cost of 27€/hour. So, the **presential meetings would cost 54€/month**, assuming that the meetings take one hour. Furthermore, we will need to **travel for pharmacies for sales and to provide technical assistance**. For that, we want to make a contract with a car renting company - LeasePlan - for one car. The car will be electric – Smart Fortwo - for a price of **520€/month**. Once more, we did not have the budget to estimate correctly how much **biological and optical material will cost per month**,

since we did not run any tests or build a prototype. So, we assume that **biological material** will cost **at least 1,394 €/month** and **optical material at least 1,000€/month**.

Finally for Marketing it will be important to develop **a website and App with information about the biosensor and the places where it can be found**, so a website domain must be paid per month. The domain would be *biosenza.com* which would cost about 1 €/month, according to Google Domains. As for the App, we would host it in Google Play and App Store, to cover both Android and iOS users. Google Play requires a one-time payment fee of about **21€**. App Store requires an annual fee of about 83€, which would cost **6.92€/month**. Internet Ads will also be important to present our product to people. In a first stage, we would like to advertise **in YouTube and sapo.pt**. We chose YouTube because it reaches a large portion of people and allows us to show how the testing works through video, which is more engaging than an image with the instructions. This would require contracting a company to do a promotional video, adding an one-time cost. Sapo.pt is a Portuguese website with news which many people access daily, considered a digital audience leader in Portugal. For YouTube we would start with an initial budget of **8.43 €/month** and sapo.pt **60€/month** for Leaderboard type of ad.

It would be also important to **advertise our biosensor in public places with posters**. The posters would be printed in vinyl paper A3 size which would cost 5.93€ in Staples Copy & Print. This price, however, could be negotiated since we are aiming to print a high quantity of posters to distribute across Portugal. Thus, we considered to print on average **50 posters per month**. The number of advertisements will vary across months, since it will depend on the revenue streams and if they are being profitable enough.

			Basis for Assumptions	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Human Resources			**Base Unit Cost per month** *unit = 1 full-time Salary or fee*	**enter # of HR units/month, not €**											
1	*Biomedical Engineer*		**2 310**	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
2	*Physics Engineer*		**2 000**	2,0	2,0	2,0	2,0	2,0	2,0	3,0	3,0	3,0	3,0	3,0	3,0
3	*Chemical Engineer*		**1 278**	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0
4	*App Developer*		**1 295**	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
5	*Product Marketing Manager*		**1 293**	0,0	0,0	0,0	0,0	0,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
6	*Sales Manager*		**1 219**	0,0	0,0	0,0	0,0	0,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0
	Number of FTEs (Full-Time Equivalents)			11,0	11,0	11,0	11,0	11,0	13,0	14,0	14,0	14,0	14,0	14,0	14,0
Revenue hypothesis		**Unit Price** of Product	Type of Unit	**Enter Revenue per month**											
	Enter Type of Product or service	or service		**(in units of revenue, not €)**											
A	*Cards/Tests*	**25**	*Cartridge*	100	100	100	100	100	150	150	150	150	150	150	150
B	*Subscription fee*	**500**	*Fee*	5	5	5	5	5	12	12	12	12	12	12	12
C	*Biosensor*	**5 000**	*Product*	10	0	0	0	0	20	0	0	0	0	0	0
D	*etc.*			0	0	0	0	0	0	0	0	0	0	0	0
E	*etc.*			0	0	0	0	0	0	0	0	0	0	0	0
	Total revenues			**55 000**	**5 000**	**5 000**	**5 000**	**5 000**	**109 750**	**9 750**	**9 750**	**9 750**	**9 750**	**9 750**	**9 750**
				Cost per month											
	DIRECT VARIABLE COSTS	**Direct cost per unit** of product													
A	*Cards/Tests*	**25**		2 500	2 500	2 500	2 500	2 500	3 750	3 750	3 750	3 750	3 750	3 750	3 750
B	*Subscription fee*	**0**		0	0	0	0	0	0	0	0	0	0	0	0
C	*Biosensor*	**6 000**		60 000	0	0	0	0	120 000	0	0	0	0	0	0
D	*etc.*			0	0	0	0	0	0	0	0	0	0	0	0
E	*etc.*			0	0	0	0	0	0	0	0	0	0	0	0
	Total direct costs			**62 500**	**2 500**	**2 500**	**2 500**	**2 500**	**123 750**	**3 750**	**3 750**	**3 750**	**3 750**	**3 750**	**3 750**
GROSS PROFIT				**-7 500**	**2 500**	**2 500**	**2 500**	**2 500**	**-14 000**	**6 000**	**6 000**	**6 000**	**6 000**	**6 000**	**6 000**
	FIXED COSTS														
A	**Human Resources**														
	Fixed remunerations			17 617	17 617	17 617	17 617	17 617	20 129	22 129	22 129	22 129	22 129	22 129	22 129
	Index and accumulated salary increase	**0,00%**		0	0	0	0	0	0	0	0	0	0	0	0
	Bonus	**0,00%**	of turnover	0	0	0	0	0	0	0	0	0	0	0	0
	Subtotal			*17 617*	*17 617*	*17 617*	*17 617*	*17 617*	*20 129*	*22 129*	*22 129*	*22 129*	*22 129*	*22 129*	*22 129*
	Total Human Resources			**17 617**	**17 617**	**17 617**	**17 617**	**17 617**	**20 129**	**22 129**	**22 129**	**22 129**	**22 129**	**22 129**	**22 129**

B **Training expenses**													
Training expenses			2 000	2 000	2 000	0	0	0	0	0	0	0	0	0
Total Training expenses			**2 000**	**2 000**	**2 000**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
C **Services rendered by third parties**														
Logistics and Delivery	**700**	per month	700	700	700	700	700	700	700	700	700	700	700	700
		per month	0	0	0	0	0	0	0	0	0	0	0	0
		per month	0	0	0	0	0	0	0	0	0	0	0	0
		per month	0	0	0	0	0	0	0	0	0	0	0	0
Total Services rendered by third parties			**700**	**700**	**700**	**700**	**700**	**700**	**700**	**700**	**700**	**700**	**700**	**700**
D **Infrastructure and operational costs**														
Laboratory Outsourcing	**216**	per month	216	216	216	216	216	216	216	216	216	216	216	216
Manufacturing running costs	**10 000**	per month	10 000	10 000	10 000	10 000	10 000	10 000	10 000	10 000	10 000	10 000	10 000	10 000
Meeting Room Rental	**54**	per month	54	54	54	54	54	54	54	54	54	54	54	54
Travels to pharmacies	**520**	per month	520	520	520	520	520	520	520	520	520	520	520	520
Biological material	**1 394**	per month	1 394	1 394	1 394	1 394	1 394	1 394	1 394	1 394	1 394	1 394	1 394	1 394
Optical material	**1 000**	per month	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
		per month	0	0	0	0	0	0	0	0	0	0	0	0
		per month	0	0	0	0	0	0	0	0	0	0	0	0
Total Infrastructure and operational costs			**13 184**	**13 184**	**13 184**	**13 184**	**13 184**	**13 184**	**13 184**	**13 184**	**13 184**	**13 184**	**13 184**	**13 184**
E **Marketing**														
Website Domain	**1**	per month	1	1	1	1	1	1	1	1	1	1	1	1
Posters	**297**	per month	297	297	297	297	297	297	297	297	297	297	297	297
Internet Ads	**68**	per month	68	68	68	68	68	68	68	68	68	68	68	68
App	**7**	per month	7	7	7	7	7	7	7	7	7	7	7	7
		per month	0	0	0	0	0	0	0	0	0	0	0	0
Total Marketing			**373**	**373**	**373**	**373**	**373**	**373**	**373**	**373**	**373**	**373**	**373**	**373**
SUMMARY														
Total revenues			55 000	5 000	5 000	5 000	5 000	109 750	9 750	9 750	9 750	9 750	9 750	9 750
Total direct costs			62 500	2 500	2 500	2 500	2 500	123 750	3 750	3 750	3 750	3 750	3 750	3 750
Total gross margin			**-7 500**	**2 500**	**2 500**	**2 500**	**2 500**	**-14 000**	**6 000**	**6 000**	**6 000**	**6 000**	**6 000**	**6 000**
Total Human Resources			17 617	17 617	17 617	17 617	17 617	20 129	22 129	22 129	22 129	22 129	22 129	22 129
Total Training expenses			2 000	2 000	2 000	0	0	0	0	0	0	0	0	0
Total Services rendered by third parties			700	700	700	700	700	700	700	700	700	700	700	700
Total Infrastructure and operational costs			13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184
Total Marketing			373	373	373	373	373	373	373	373	373	373	373	373
Total costs (excluding depreciations)			**33 874**	**33 874**	**33 874**	**31 874**	**31 874**	**34 386**	**36 386**	**36 386**	**36 386**	**36 386**	**36 386**	**36 386**
Operating Profit excl. depreciations			**-41 374**	**-31 374**	**-31 374**	**-29 374**	**-29 374**	**-48 386**	**-30 386**	**-30 386**	**-30 386**	**-30 386**	**-30 386**	**-30 386**

D.2 – NWC

	Assumptions		Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Clients (/product)														
Total revenues			55 000	5 000	5 000	5 000	5 000	109 750	9 750	9 750	9 750	9 750	9 750	9 750
Suppliers (/item)														
DIRECT VARIABLE COSTS			62 500	2 500	2 500	2 500	2 500	123 750	3 750	3 750	3 750	3 750	3 750	3 750
Human Resources			17 617	17 617	17 617	17 617	17 617	20 129	22 129	22 129	22 129	22 129	22 129	22 129
Training expenses			2 000	2 000	2 000	0	0	0	0	0	0	0	0	0
Services rendered by third parties			700	700	700	700	700	700	700	700	700	700	700	700
Infrastructure and operational costs			13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184
Marketing			373	373	373	373	373	373	373	373	373	373	373	373
CALCULATION														
	100% = 3 months of stock													
Stock and work in progress	0	**100%** of direct var costs	62 500	65 000	67 500	7 500	7 500	128 750	130 000	131 250	11 250	11 250	11 250	11 250
Clients: customer credit	**100%= 3 months of customer credit**													
Total revenues	0	**100%** of turnover	55 000	60 000	65 000	15 000	15 000	119 750	124 500	129 250	29 250	29 250	29 250	29 250
Subtotal	0		55 000	60 000	65 000	15 000	15 000	119 750	124 500	129 250	29 250	29 250	29 250	29 250
Supplier debt	**100%= 3 months of suppliers credit**													
DIRECT VARIABLE COSTS	**33%**	of costs	20 625	21 450	22 275	2 475	2 475	42 488	42 900	43 313	3 713	3 713	3 713	3 713
Human Resources	**0%**	of costs	0	0	0	0	0	0	0	0	0	0	0	0
Training expenses	**33%**	of costs	660	1 320	1 980	1 320	660	0	0	0	0	0	0	0
Services rendered by third parties	**33%**	of costs	231	462	693	693	693	693	693	693	693	693	693	693
Infrastructure and operational costs	**33%**	of costs	4 351	8 702	13 053	13 053	13 053	13 053	13 053	13 053	13 053	13 053	13 053	13 053
Marketing	**33%**	of costs	123	246	369	369	369	369	369	369	369	369	369	369
Subtotal	0		21 516	23 232	24 948	4 488	3 828	43 181	43 593	44 006	4 406	4 406	4 406	4 406
Working capital need	0		95 984	101 768	107 552	18 012	18 672	205 320	210 907	216 495	36 095	36 095	36 095	36 095
TRANSFER TO BALANCE SHEET														
Raw materials and work in progress			62 500	65 000	67 500	7 500	7 500	128 750	130 000	131 250	11 250	11 250	11 250	11 250
Accounts receivable			55 000	60 000	65 000	15 000	15 000	119 750	124 500	129 250	29 250	29 250	29 250	29 250
Accounts payable			21 516	23 232	24 948	4 488	3 828	43 181	43 593	44 006	4 406	4 406	4 406	4 406
working capital need			**95 984**	**101 768**	**107 552**	**18 012**	**18 672**	**205 320**	**210 907**	**216 495**	**36 095**	**36 095**	**36 095**	**36 095**
TRANSFER TO TREASURY														
Change in working capital need			95 984	5 784	5 784	-89 540	660	186 648	5 588	5 588	-180 400	0	0	0

D.3 – Investments

The investments on tangible fixed assets that will be done in the first month are computers for the employees and lab materials. After launching the pilot version of the biosensor, in month 6, we expect to make an investment on a manufacturing facility as well as manufacturing equipment.

				Date #:	1	2	3	4	5	6	7	8	9	10	11	12
					Month 1	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**	**Month 7**	**Month 8**	**Month 9**	**Month 10**	**Month 11**	**Month 12**
INVESTMENTS																
TANGIBLE FIXED ASSETS	**Amount**	**Date**	**Depreciation time**													
Computers	**8 000**	**1**	**6**	year	8 000	0	0	0	0	0	0	0	0	0	0	0
Lab material	**50 000**	**1**	**15**	year	50 000	0	0	0	0	0	0	0	0	0	0	0
Manufacturing Facility	**500 000**	**6**	**20**	year	0	0	0	0	0	500 000	0	0	0	0	0	0
Manufacturing Equipment	**2 000 000**	**6**	**20**	year	0	0	0	0	0	2 000 000	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
				year	0	0	0	0	0	0	0	0	0	0	0	0
,,,				year	0	0	0	0	0	0	0	0	0	0	0	0
Total					58 000	0	0	0	0	2 500 000	0	0	0	0	0	0
Accumulated total					*58 000*	*58 000*	*58 000*	*58 000*	*58 000*	*2 558 000*	*2 558 000*	*2 558 000*	*2 558 000*	*2 558 000*	*2 558 000*	*2 558 000*
Total to be paid					58 000	0	0	0	0	2 500 000	0	0	0	0	0	0

TRANSFER TO BALANCE SHEET												
Book value (cost less depreciation)												
Tangible Fixed Assets	57 611	57 222	56 833	56 444	56 056	2 545 250	2 534 444	2 523 639	2 512 833	2 502 028	2 491 222	2 480 417
TRANSFER TO P&L STATEMENT												
Total Depreciations	-389	-389	-389	-389	-389	-10 806	-10 806	-10 806	-10 806	-10 806	-10 806	-10 806
TRANSFER TO TREASURY												
Investment payments	-58 000	0	0	0	0	-2 500 000	0	0	0	0	0	0

D.4 – Finance

In terms of equity, we want to start the shares seed round with 800,000 shares at 5€ per share in the first month. Later on the 6th month, we plan to offer another 500,000 shares at 5€ per share. As for equity subsidies, we look to participate in a Start-up Acceleration competition organized by BGI with a prize of 5,000€ in the first month.

				1	2	3	4	5	6	7	8	9	10	11	12
				Month 1	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**	**Month 7**	**Month 8**	**Month 9**	**Month 10**	**Month 11**	**Month 12**
EQUITY															
	Number	Price	Date (column #)												
Shares seed round	**800 000**	**5**	**1**	4 000 000	0	0	0	0	0	0	0	0	0	0	0
Shares round 1	**500 000**	**5**	**6**	0	0	0	0	0	2 500 000	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
Total				4 000 000	0	0	0	0	2 500 000	0	0	0	0	0	0
EQUITY SUBSIDIES	Amount (€)	Date (col. #)													
BGI Grant	**5 000**	**1**		5 000	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	0	0	0
Total Subsidies				5 000	0	0	0	0	0	0	0	0	0	0	0
DEBT															
	Amount (€)	Date (column #)	Duration	Annual interest	Repayment starts after:										
SHORT-TERM DEBT: duration ≤ 1 year															
Enter name of Lender or Loan		1	1,0 years	0,0%	1 months	0	0	0	0	0	0	0	0	0	0	0	0
		1	0,5 years	0,0%	1 months	0	0	0	0	0	0	0	0	0	0	0	0
		1	1,0 years	0,0%	1 months	0	0	0	0	0	0	0	0	0	0	0	0
		1	1,0 years	0,0%	1 months	0	0	0	0	0	0	0	0	0	0	0	0
		1	1,0 years	0,0%	1 months	0	0	0	0	0	0	0	0	0	0	0	0
LONG-TERM DEBT: duration > 1 year															
Enter name of Lender or Loan		1	2 years	5,0%	1 months	0	0	0	0	0	0	0	0	0	0	0	0
		1	5 years	0,0%	1 months	0	0	0	0	0	0	0	0	0	0	0	0
		1	years	0,0%	1 months	0	0	0	0	0	0	0	0	0	0	0	0
		1	years	0,0%	1 months	0	0	0	0	0	0	0	0	0	0	0	0
		1	years	0,0%	1 months	0	0	0	0	0	0	0	0	0	0	0	0
Total						0	0	0	0	0	0	0	0	0	0	0	0
Accumulated						**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
total debt						0	0	0	0	0	0	0	0	0	0	0	0
TRANSFER TO BALANCE SHEET															
Share Capital				0	4 000 000	4 000 000	4 000 000	4 000 000	4 000 000	6 500 000	6 500 000	6 500 000	6 500 000	6 500 000	6 500 000	6 500 000	6 500 000
Capital grants					5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000
debts payable after 1 year					0	0	0	0	0	0	0	0	0	0	0	0	0
Debts payable within the year					0	0	0	0	0	0	0	0	0	0	0	0	0
TRANSFER TO TREASURY															
Shares issued					4 000 000	0	0	0	0	2 500 000	0	0	0	0	0	0	0
Capital subsidies					5 000	0	0	0	0	0	0	0	0	0	0	0	0
Proceeds from Loans					0	0	0	0	0	0	0	0	0	0	0	0	0
Interest payments					0	0	0	0	0	0	0	0	0	0	0	0	0
Repayment of loan capital					0	0	0	0	0	0	0	0	0	0	0	0	0

D.5 – P&L

Turnover Assumptions		Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Cards/Tests		2 500	2 500	2 500	2 500	2 500	3 750	3 750	3 750	3 750	3 750	3 750	3 750
Subscription fee		2 500	2 500	2 500	2 500	2 500	6 000	6 000	6 000	6 000	6 000	6 000	6 000
Biosensor		50 000	0	0	0	0	100 000	0	0	0	0	0	0
etc.		0	0	0	0	0	0	0	0	0	0	0	0
etc.		0	0	0	0	0	0	0	0	0	0	0	0
Turnover		55 000	5 000	5 000	5 000	5 000	109 750	9 750	9 750	9 750	9 750	9 750	9 750
- Direct , variable costs		62 500	2 500	2 500	2 500	2 500	123 750	3 750	3 750	3 750	3 750	3 750	3 750
Gross margin		-7 500	2 500	2 500	2 500	2 500	-14 000	6 000	6 000	6 000	6 000	6 000	6 000
- Fixed costs													
Human Resources		17 617	17 617	17 617	17 617	17 617	20 129	22 129	22 129	22 129	22 129	22 129	22 129
Training expenses		2 000	2 000	2 000	0	0	0	0	0	0	0	0	0
Services rendered by third parties		700	700	700	700	700	700	700	700	700	700	700	700
Infrastructure and operational costs		13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184	13 184
Marketing		373	373	373	373	373	373	373	373	373	373	373	373
Subtotal		*33 874*	*33 874*	*33 874*	*31 874*	*31 874*	*34 386*	*36 386*	*36 386*	*36 386*	*36 386*	*36 386*	*36 386*
- Depreciation		389	389	389	389	389	10 806	10 806	10 806	10 806	10 806	10 806	10 806
Operating Profit (EBIT)		-41 763	-31 763	-31 763	-29 763	-29 763	-59 192	-41 192	-41 192	-41 192	-41 192	-41 192	-41 192
Interest charges		0	0	0	0	0	0	0	0	0	0	0	0
Profit after Financial costs		-41 763	-31 763	-31 763	-29 763	-29 763	-59 192	-41 192	-41 192	-41 192	-41 192	-41 192	-41 192
Exceptional income or (-costs)		0											
Profit before tax		-41 763	-31 763	-31 763	-29 763	-29 763	-59 192	-41 192	-41 192	-41 192	-41 192	-41 192	-41 192
Tax charge	**17%**	0	0	0	0	0	0	0	0	0	0	0	0
Net Profit		-41 763	-31 763	-31 763	-29 763	-29 763	-59 192	-41 192	-41 192	-41 192	-41 192	-41 192	-41 192
TRANSFER TO BALANCE SHEET													
Accumulated profit after taxes	0	-41 763	-73 526	-105 290	-135 053	-164 816	-224 008	-265 200	-306 392	-347 584	-388 776	-429 967	-471 159

D.6 – Cash Flow: Treasury

		Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Operating profit		-41 763	-31 763	-31 763	-29 763	-29 763	-59 192	-41 192	-41 192	-41 192	-41 192	-41 192	-41 192
+ Depreciations		389	389	389	389	389	10 806	10 806	10 806	10 806	10 806	10 806	10 806
- Change in NWCN		-95 984	-5 784	-5 784	89 540	-660	-186 648	-5 588	-5 588	180 400	0	0	0
Cash Flow from operations		-137 358	-37 158	-37 158	60 166	-30 034	-235 034	-35 974	-35 974	150 014	-30 386	-30 386	-30 386
- Exceptional income or costs		0	0	0	0	0	0	0	0	0	0	0	0
- Taxes		0	0	0	0	0	0	0	0	0	0	0	0
Total auto financing		-137 358	-37 158	-37 158	60 166	-30 034	-235 034	-35 974	-35 974	150 014	-30 386	-30 386	-30 386
- Investments		-58 000	0	0	0	0	-2 500 000	0	0	0	0	0	0
- Interest on long term debt		0	0	0	0	0	0	0	0	0	0	0	0
- Reimbursements		0	0	0	0	0	0	0	0	0	0	0	0
Amount to be financed		-195 358	-37 158	-37 158	60 166	-30 034	-2 735 034	-35 974	-35 974	150 014	-30 386	-30 386	-30 386
+ Share capital		4 000 000	0	0	0	0	2 500 000	0	0	0	0	0	0
+ Capital subsidies		5 000	0	0	0	0	0	0	0	0	0	0	0
+ Debt		0	0	0	0	0	0	0	0	0	0	0	0
+ Interest on overdraft	0,00%	0	0	0	0	0	0	0	0	0	0	0	0
Cash Flow		3 809 642	-37 158	-37 158	60 166	-30 034	-235 034	-35 974	-35 974	150 014	-30 386	-30 386	-30 386
TREASURY POSITION	0	**3 809 642**	**3 772 483**	**3 735 325**	**3 795 491**	**3 765 456**	**3 530 423**	**3 494 449**	**3 458 475**	**3 608 489**	**3 578 102**	**3 547 716**	**3 517 330**
TRANSFER TO BALANCE SHEET													
Available liquidity		3 809 642	3 772 483	3 735 325	3 795 491	3 765 456	3 530 423	3 494 449	3 458 475	3 608 489	3 578 102	3 547 716	3 517 330
TRANSFER TO P/L STATEMENT													
Interest		0	0	0	0	0	0	0	0	0	0	0	0

D.7 – Balance Sheet

		Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
ASSETS													
Tangible fixed assets		57 611	57 222	56 833	56 444	56 056	#######	#######	#######	2 512 833	2 502 028	2 491 222	2 480 417
Stocks and Work in Progress		62 500	65 000	67 500	7 500	7 500	128 750	130 000	131 250	11 250	11 250	11 250	11 250
Accounts receivable		55 000	60 000	65 000	15 000	15 000	119 750	124 500	129 250	29 250	29 250	29 250	29 250
Cash		#######	#######	#######	#######	#######	#######	#######	#######	3 608 489	3 578 102	3 547 716	3 517 330
Total assets		#######	#######	#######	#######	#######	#######	#######	#######	***6 161 822***	***6 120 630***	***6 079 438***	***6 038 246***
EQUITY AND LIABILITIES													
Share Capital		#######	#######	#######	#######	#######	#######	#######	#######	6 500 000	6 500 000	6 500 000	6 500 000
Capital subsidies		5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000	5 000
Accumulated P/L		-41 763	-73 526	-105 290	-135 053	-164 816	-224 008	-265 200	-306 392	-347 584	-388 776	-429 967	-471 159
Debts payable after 1 year		0	0	0	0	0	0	0	0	0	0	0	0
Debts payable within 1 year		0	0	0	0	0	0	0	0	0	0	0	0
Accounts payable		21 516	23 232	24 948	4 488	3 828	43 181	43 593	44 006	4 406	4 406	4 406	4 406
Overdraft		0	0	0	0	0	0	0	0	0	0	0	0
Total equity and liabilities	0	#######	#######	#######	#######	#######	#######	#######	#######	***6 161 822***	***6 120 630***	***6 079 438***	***6 038 246***
Balance (should be 0)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
(Assets – Equity & Liabilities)													

D.8 – Summary

All values are in Euros.

BALANCE SHEET	sem 1, year 1	sem 2, year 1
ASSETS		
Tangible fixed assets	2 545 250	2 480 417
Stock and work in progress	128 750	11 250
Accounts receivable	119 750	29 250
Cash	3 530 423	3 517 330
Total assets	**6 324 173**	**6 038 246**
EQUITY AND LIABILITIES		
Share Capital	6 500 000	6 500 000
Capital subsidies	5 000	5 000
Accumulated P/L	-224 008	-471 159
Debts payable after 1 year	0	0
Debts payable within 1 year	0	0
Accounts payable	43 181	4 406
Negative treasury	0	0
Total equity and liabilities	**6 324 173**	**6 038 246**

P&L STATEMENT	sem 1, year 1	sem 2, year 1
Cards/Tests	16 250	22 500
Subscription fee	18 500	36 000
Biosensor	150 000	0
etc.	0	0
etc.	0	0
Turnover	**184 750**	**58 500**
- Direct , variable costs	196 250	22 500
Gross margin	**-11 500**	**36 000**
Human Resources	108 214	132 774
Training expenses	6 000	0
Services rendered by third parties	4 200	4 200
Infrastructure and operational costs	79 107	79 107
Marketing	2 237	2 237
- Depreciation	12 750	64 833
Operating Profit	**-224 008**	**-247 151**
Financial charges	0	0
Exceptional costs	0	0
Profit before tax	**-224 008**	**-247 151**
Tax	0	0
Net Profit	**-224 008**	**-247 151**

TREASURY POSITION	sem 1, year 1	sem 2, year 1
Operational result	-224 008	-247 151
+ Depreciations	12 750	64 833
- Change in net working capital need	-205 320	169 225
Cash Flow from operations	**-416 577**	**-13 093**
- Reimbursements	0	0
- Interest	0	0
- Investments	-2 558 000	0
- Taxes	0	0
+ Share capital	6 500 000	0
+ Capital subsidies	5 000	0
+ Debt	0	0
Cash Flow	**3 530 423**	**-13 093**
TREASURY POSITION	3 530 423	3 517 330