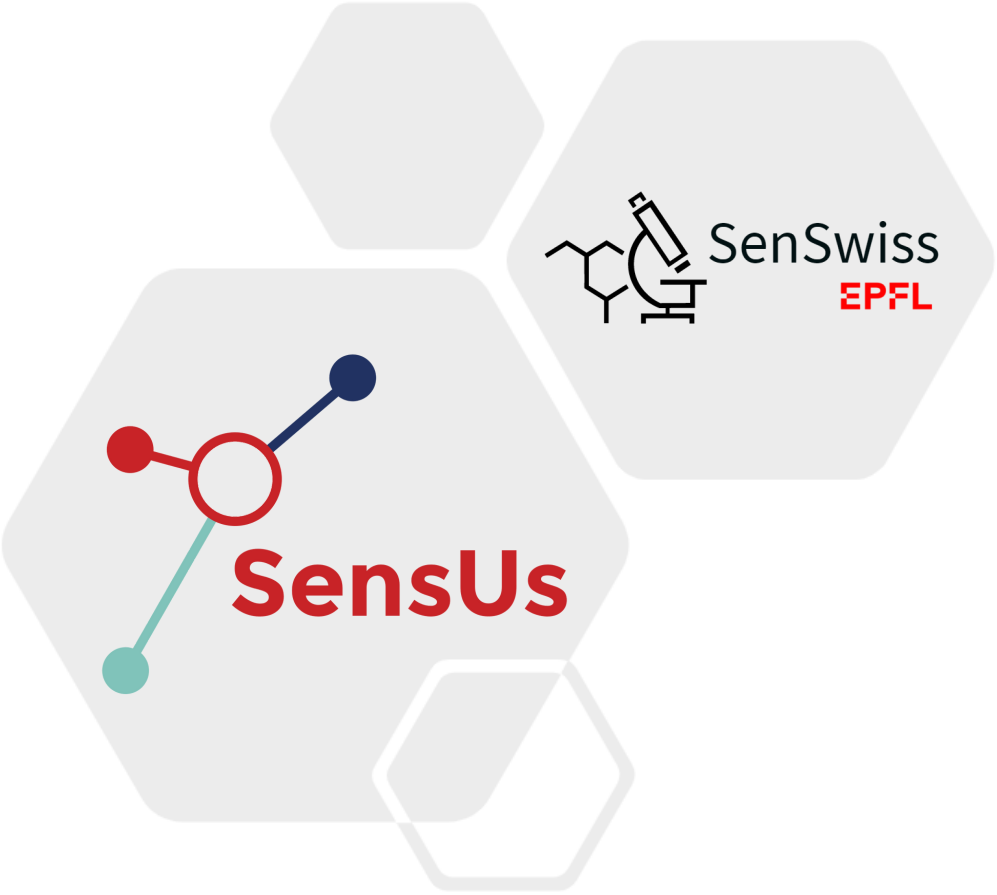
Team Results Document



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August 17th 2021

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"The other two-thirds stayed firmly at home and lived full, rich and happy lives until they were all suddenly wiped out by a virulent disease contracted from a dirty telephone."

Douglas Adams
The Hitchhiker's Guide to the Galaxy

1 SUMMARY

Our team developed a biosensor for the rapid quantification of hemagglutinin-1 (H1) in saliva. H1 is the main surface protein of the influenza virus. The current COVID pandemic has made clear the danger that transmittable respiratory diseases pose. In this context, immuno-based rapid influenza diagnostic tests are very promising, however, these often lack sensitivity and are not universally accessible. To suppress upcoming pandemics and for patients at risk, such as the elderly and pregnant women, this is problematic. To tackle this issue, we developed an easy-to-use biosensor, able to rapidly and reliably quantify the concentration of H1 in saliva. Our biosensor uses the Extraordinary Optical Transmission phenomenon. By using a nanohole array functionalized with anti-H1 antibodies, and functionalized gold nanoparticles, detection of H1 can be performed. Through the monitoring of the locally transmitted light, we detect single-binding-events, which is indicative of the H1 concentration. Our vision is to make rapid testing universally accessible. Automatic Testing Machines (ATM) will be strategically positioned in public spaces, enabling anyone to perform self-tests. We believe that democratizing biosensors can have great social impact by reducing the overload of healthcare professionals and improving universal access to testing of diseases which require an urgent diagnosis.

2 BIOSENSOR SYSTEM AND ASSAY

2.1 Molecular recognition and assay reagents

Our biosensor is a protein-based sandwich assay, using two different antibodies to bind the distinct parts of the target ligand, here hemagglutinin (H1). Both antibodies are monoclonal, to ensure higher specificity to the H1 strain we want to detect. First, capture antibodies (11055-RM05 Sinobiological) are spotted at precise locations on a gold nanohole array (AU-NHAs) surface and adhere to it physically (figure 1A). Then, the surface is passivated with Clear Milk Blocking Buffer to avoid nonspecific binding of molecules to the non-spotted surface. Next, the saliva sample is centrifuged at 11k rpm for 1 minute [1] for the removal of the mucin proteins. The supernatant is then mixed with the reaction buffer composed with phosphate buffer saline (PBS), NaOH (50mM) and Tween20 (2.5%). This buffer will increase the pH of the sample to around 9, ensuring the best interaction of the antibodies with the analyte, and limit non-specific interactions. Then, 100nm diameter gold nanoparticles (AU-NP) covered with a detection antibody (11055-MM04T Sinobiological) are mixed into the diluted sample and flowed over the gold surface (Figure 1B). The detection antibodies will bind to H1 molecules, and to the capture antibodies at the surface. Upon shining light through the AU-NHAs surface, the light intensity is locally decreased where there is binding of the AU NP to the ligand, allowing the measurement of binding events (see Figure 1C).

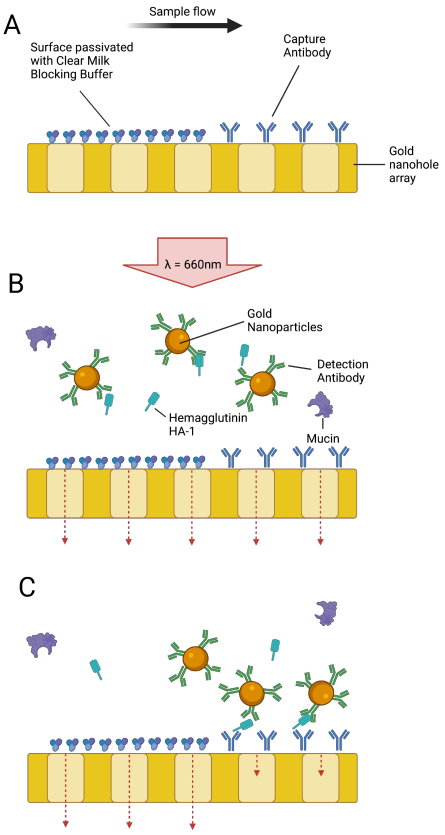


Figure 1: Scheme of the molecular recognition sandwich assay.
A: The gold nanohole array after being spotted with the capture antibodies and passivated with the blocking buffer.
B: The saliva sample with the reaction buffer mix is flowed over the surface.
C: Upon the presence of the analyte, here H1, there is binding of the AU-NP to the capture antibodies through the molecule of interest, allowing the detection of H1 and the measurement of its concentration.

2.2 Physical transduction

To enable the detection of H1 molecules in saliva, we use an optical transduction mechanism: a plasmonic-based sensing method known as nanoparticle-enhanced plasmonic gold nanohole array sensing. When excited at a 660 nm wavelength the AU-NHA transduces approximately 2% of the incoming light through its subwavelength holes. This plasmonic phenomenon is known as extraordinary optical transmission (EOT). It exhibits uniform transmission without the presence of a sample. Nevertheless, upon binding of the AU-NP to the ligand on the surface, the EOT in the neighboring holes is heavily perturbed. This effectively results in a locally decreased intensity which can be visualized using a simple CMOS camera and used to detect single-binding-events of single AU-NP to the surface.

2.3 Cartridge technology

The AU-NHAs are made of silicon oxide covered with a titanium adhesion layer and a gold layer. The holes have dimensions of 200nm in diameter with a periodicity of 600nm. The capture antibodies are spotted by inkjet printing with the sciFLEXARRAYER S3 Scienion. The size of the spots varies from 150 to 170 µm.

The AU-NHA chip is inserted and fixed inside a cartridge that contains fluidic elements, enabling an easier manipulation. The cartridge itself is made out of 2 layers of PMMA of 20 x 75 mm. The bottom layer is 0.5 mm thick, the same thickness as the chip. It contains a hole that is exactly the same dimensions as that of the chip (9.8x9.8mm). The gold chip is inserted in this hole, then the PMMA and chip are fixed together using adhesive vinyl that also plugs the gap between them to avoid leaks. On top of this PMMA and gold layer, a double-sided adhesive layer including a channel is used to tape it together. The channel is cut in the adhesive layer, and its dimensions are 45 mm x 1.6 mm x 100 µm, so its volume is smaller than 10 µL. Finally, the top layer is deposited on the double-sided adhesive layer, and it contains one inlet and one outlet of 2 mm diameter, one of which is assembled with an O-ring for connection with a small pipe. The blocking buffer as well as the sample mixed with the reaction buffer are flowed in and out of the channel using a pump, connected with pipes. For the reading step, the cartridge is placed inside a holder, and simultaneously the camera is placed just under the AU-NHA chip to record the binding events while flowing the sample.

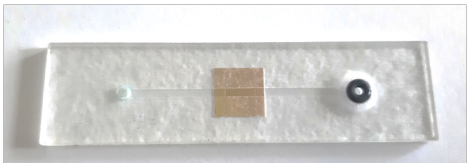


Figure 2: Photo of the assembled cartridge. The AU-NHA chip can be observed at the middle. On the right, the O-ring can be seen, which will do the connection to the pipes of the pump.

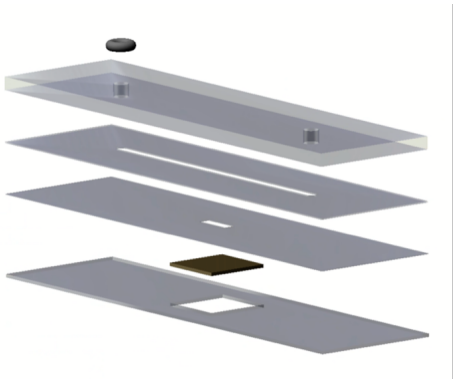


Figure 3: CAD model of the different layers of the cartridge. From top to bottom: O-ring, top PMMA layer, channel made using double sided-tape, adhesive vinyl, Au-NHA, bottom PMMA layer

For now, a laboratory syringe pump is used to flow the sample as well as the blocking buffer over the AU-NHA. We are working on integrating the AMF SPM pump inside of our device to reduce the footprint of the device.

2.4 Reader instrument and user interaction

The reader instrument measures 25cm $\times$ 25cm $\times$ 40cm and is composed of two parts. First, an optical setup is used to induce the EOT phenomena and measure the AU-NP binding through the CMOS camera. This setup (Figure 4 and Figure S1) comprises a LED whose light is homogenized by a lens. A 660 nm wavelength band-pass filter selects the specific wavelength used to induce the EOT. At the bottom of the AU-NHA surface, a CMOS camera is placed to measure the difference in light intensity and translate this into concentration measurement. The cartridge is inserted at the middle of the system by lifting the top part of the biosensor. It is placed into a cartridge holder which fixes its position relative to the CMOS camera. This setup allows for live measurements to be taken. The focus is set to the AU-NP and can easily be adjusted. At the back of the optical setup, a pump will be placed which allows the flow of the blocking buffer and the sample in a precise and controlled manner. For now, an external syringe pump is used but we are working on integrating a pump inside of our device for a seamless experience. The second part of the reader instrument consists of a base support containing the power supply and a Raspberry Pi programmed to allow user-machine interaction. Through this interface, it is possible to start a new measurement and visualize the result of the test, i.e., if H1 was detected and the corresponding viral load.

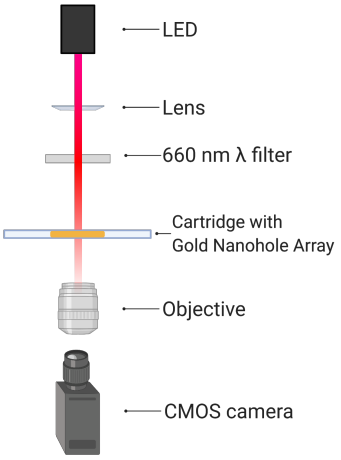


Figure 4: Simplified scheme of the optical setup used to induce EOT and measure the binding of the AU NPs.

For now, the user can manually select the regions of interest (ROI) on the AU-NHA to be taken into consideration while measuring the concentration. Nevertheless, this step is aimed to be automatized in the future (see section 7).

3 TECHNOLOGICAL FEASIBILITY

While developing our sensor, the first step was to verify the molecular recognition by testing the protein-antibody interaction and the effect of the saliva matrix. Three different pairs of anti-H1 antibodies were tested using an EOT-setup similar to ours, to determine the couple which provided the highest signal-to-noise ratio. Ultimately, the antibodies 11055-RM05 and 1105-MM04T (Sinobiological) were determined to be the best capture and detection antibodies pair, respectively. Both of these antibodies being monoclonal, they assure a highly specific detection of H1.

Regarding the saliva matrix, different protocols were tested to reduce the noise induced by its high viscosity and the presence of other molecules. These included: heating, filtrating and centrifuging of the sample, this last showing the best results (see Figure 5). Using this EOT-setup combined with the sample preparation we were able to detect H1 in saliva at a concentration of 1 ng/ml (as shown in Figure 6). Subsequently, the cartridge was developed to allow a simple flow through the AU-NHA and shorten the time of detection. We are currently investigating the LOD of our setup, which based on previous experiments performed by *Alexander Belushkin* is expect it to be around 50 pg/ml [2].

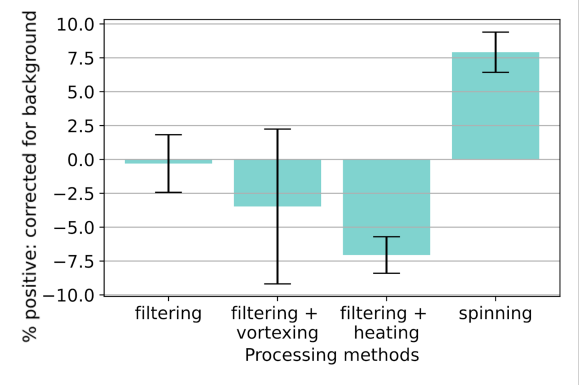


Figure 5: Signal corrected for background for different types of sample preparations

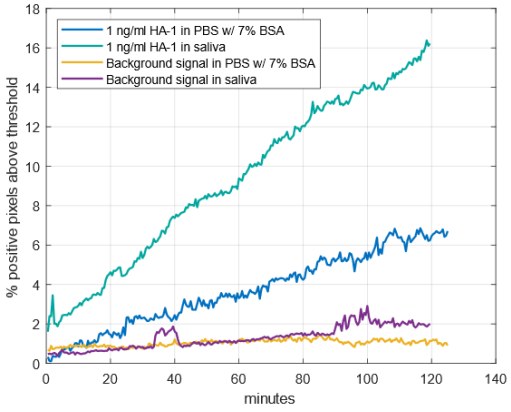


Figure 6: Live measurements of H1 in PBS and in spinned saliva.

The dimensions of the cartridge are similar to those of microscope slides to make the handling simple. Regarding the size of the channels, it was chosen as twice the field of view of the camera, giving us sufficient area to select the regions of the spotted capture antibodies. The width of the channel makes our setup robust against small misalignment made during the manual assembly of the cartridges. In the future, a channel with a smaller width could be used once manufacturing is streamlined thus increasing the number of channels per cartridge and reducing costs. As for the materials, PMMA was chosen because it is optically transparent to the wavelength used to induce SPR on the AU-NHA. Furthermore, PMMA is inexpensive, easy to manufacture during prototyping using a laser cutter and it can be used for large scale production. Iterative approaches enabled finding the best method to flow the sample in a controlled manner, which was found to be by using a syringe pump. The next step in improving our device will be to use a microfluidic pump to automate the different flows. The optimal flow was calculated considering the height of the depletion layer related to the flow rate. Basically, we aim for a flow rate high enough to allow the smallest depletion layer, so that the concentration will be uniform over the entire surface[3].

Regarding the physical transduction, the setup used is simple and allows a fast assembly of the components. The LED, the lens and the 660 nm band-pass filter were inserted into a tubing so that the LED is positioned close to the focal point of the lens. The focal distance of the CMOS camera to the cartridge was set manually, according to the focal distance given by the manufacturer, and fixed into the setup.

One of the main advantages of this sensing technology is that it can be easily adapted to detect multiple different analytes, whether they are small proteins as H1 or intact viruses. Yanik et al. [4] were able to detect both small RNA and large DNA viruses using a similar EOT-based setup, in a labeled-free manner. Similarly, Cetin et al. [5] developed a small plasmonic biosensor able to detect intact H1N1 influenza virus, although not in saliva. Indeed, it suffices to change the capture and detection antibodies for new ones specific to the target we want. To this end, preprocessing steps must be reviewed, namely, the centrifuging parameters must be adapted according to the weight and size of the H1N1 virus.

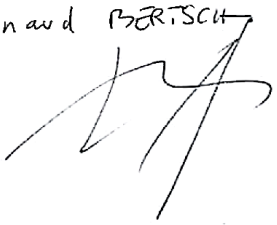
4 ORIGINALITY

Team captains: Our team developed a new cartridge and setup aiming at simplifying the manipulation and boosting the time needed to have the measurement result. By introducing microfluidics to the system we are able to provide a controlled flow rate of the sample. After careful literature research, we tested many approaches to adapt the biosensor to the saliva matrix, and ensure that the background noise due to the unspecific binding of non-H1 proteins was reduced. Furthermore, we implemented an easy-to-use readout code for image processing of the results and to provide the final concentration of the analyte based on our calibration curve. These improvements reduced possible errors from misuse and aimed at making the device user-friendly and invariant upon manipulation. Concerning the translational potential, our innovative business model came from brainstorming within the business team, after contacting doctors, pharmacies, researchers and companies. We believe this technology can be a landmark on the biosensors field by democratizing the device to the general population.

Supervisor: The biosensor developed by the EPFL team is based on the phenomenon of Extraordinary Optical Transmission (EOT). This phenomenon was first reported by Ebbesen et al. in 1998 and uses a thin metal layer patterned with a subwavelength nanohole array. When light at a specific wavelength is directed towards this patterned metal layer, enhanced transmission occurs. The transmission maximum can be shifted by changing the dielectric conditions close to the metal interface, for example when biomolecules or nanoparticles are in close proximity to this metallic nanohole structure. If the EOT principle is not new, it has for advantage its simplicity of implementation as it does not require complex optics, polarized light sources or moving parts. Until now EOT has been used in biosensors applications for the detection of viruses, bacteria and cells. Only a limited number of publications report the use of EOT for the detection of proteins and smaller molecules, with most of them using artificially made samples and working in simplified non-physiological conditions. Only Belushkin et al. used this method on actual serum samples from patients to detect two sepsis biomarker proteins. To my knowledge, EOT has never been used in biosensors using saliva samples, which is a challenge as saliva contains proteins that may induce measurement noise related to non-specific binding. The biosensor developed by the SenSwiss team is based on the work by Belushkin et al., however, this system had to be adapted to saliva rather than blood serum. It was also significantly modified and simplified to reduce the number of manual operations required to operate it. The time of analysis had also to be considerably reduced for it to be compatible with the constraints of the SensUs competition. This implied to develop a simple and robust optical system without moving parts, to select and evaluate a number of surface functionalization strategies in order to optimize and enhance the biosensor signal, to develop efficient image analysis software tools and to understand how simple microfluidics could benefit the project. Finally, a concept of a device using a microfluidic cartridge was developed providing both the ease of use and simplifying the manipulation of sample and reagents.

Lausanne, le 17 août 2021

Arnaud BERTSCH



William Verstraeten

Deborah Scherrer Ma

5 TRANSLATION POTENTIAL

5.1 Business model canvas

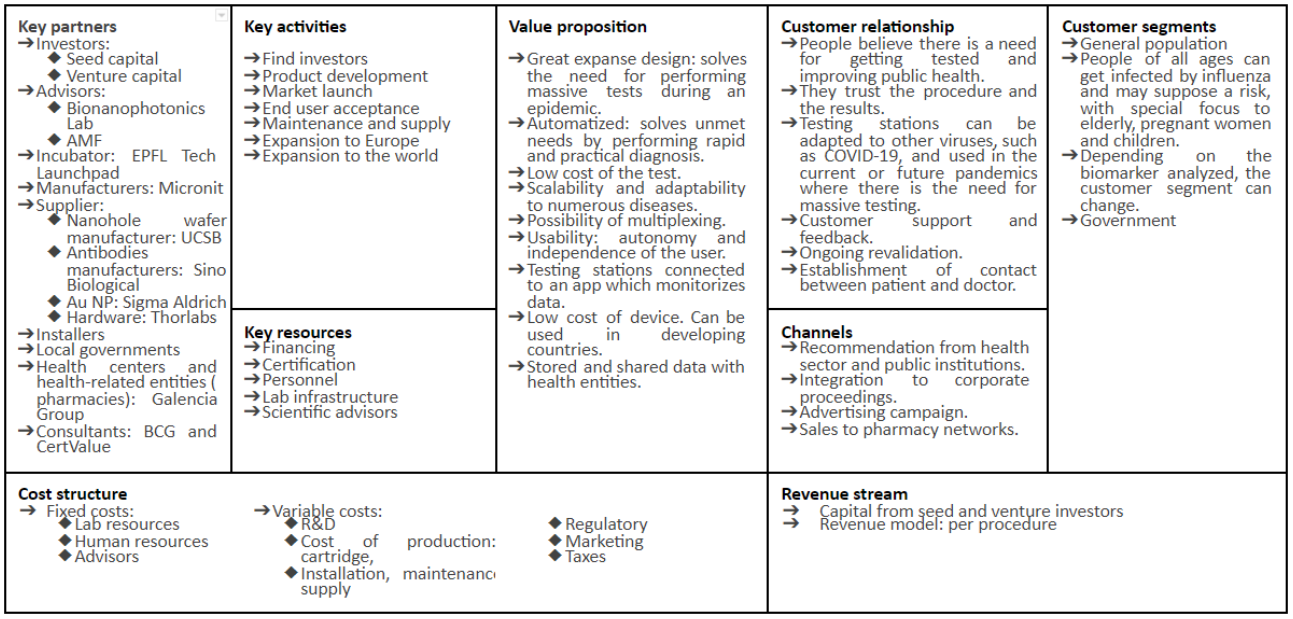


Figure 7: Business model canvas. This table can found in larger format in Appendix S6

5.2 Market description

While pandemics arise globally, the healthcare market is seeking faster, easier and more readily available technologies for testing large populations. For instance, influenza pandemics have been reported for at least 500 years, with inter-pandemic intervals averaging approximately 40 years [6] and pose a continual threat due to novel emerging strains [7]. Additionally, outbreaks of influenza are prevalent in the winter season, causing 650'000 annual deaths globally [8]. In Switzerland, the number of medical consultations concerning the flu is approximately 200'000 [9]. While people of different ages and medical histories are at risk for influenza, the major population at risk is considered to be the elderly people, pregnant women, children and people affected by chronic diseases and HIV [10]. At the moment, when having flu-like symptoms, the patient will get a doctor appointment, and be diagnosed either by visual inspection or carrying out a test such as RT-PCR or LFIA and prescribe the medication. This method is costly and time-consuming for both the patient and the healthcare system. We propose a solution based on an infrastructure of Automated Testing Machines (ATMs), spread around the cities and crowded places such as train stations and airports. With this, the customer flow is the following (Figure S2 in the Appendix). When testing conditions are identified, such as having symptoms, before going to an event or when traveling, the user will localize and go to the nearest ATM and undergo a saliva test autonomously. The medical device market is regulated by the regulation (EU) 2017/745, which applies in EU countries as well as Switzerland[11]. This regulation describes a biosensor such as the one developed by our team as a Class I medical device, and therefore has to follow the regulatory process of such device. In order to commercialize a product that fits in the medical device market, homologation and acquisition of the CE certification are necessary.

5.3 Stakeholder desirability

There is a lack of a robust infrastructure for testing that allows easy and fast access to diagnosis of a highly contagious or highly severe disease. This has been observed in the current pandemic of COVID-19, where the existing techniques are not providing a solution to this problem. For example, RT-PCR tests, in addition to being expensive, need prior reservations and scheduling in most testing centers, so is not considered as a time-efficient and sustainable solution in a patient's perspective [12, 13]. As for rapid tests, lateral flow immunoassays (LFIA) are used but they have lower sensitivity [14] and do not offer a quantitative result, which in case of an outbreak is important information about the transmission rate. Both of these techniques use nasopharyngeal

swabs, which are invasive, uncomfortable and sometimes painful for the patients. According to the GHOL Hospital Head of Laboratory, Dr. Maitrejan, these tests are only available through healthcare professionals, and patients being major stakeholders in such pathways are seeking fast solutions as well as a test that is non-invasive, painless and easy to make. On the other hand, medical professionals aim at guaranteeing reliable repeatable results. Furthermore, preparation, sampling and analyzing is a time-consuming process that needs professional handling and special training. Thus, there is a need for an affordable monitoring device that would minimize the time spent by the customer as well as healthcare professionals, in order to reduce the load on the healthcare system.

Our biosensor based on nanoplasmonic imaging technology allows a real-time, quantitative and in situ detection of the biomarkers present in saliva, which is a robust and inexpensive solution. By implementing ATMs at different locations of the cities, the user can take a test independently, autonomously and at any time of the day. This test can simply be performed with an easy-to-use saliva sample test kit, found at the ATM or at the nearest store. Apart from the freedom given to the user of not having to make an appointment, the result is sent in less than 15 minutes, which represents a gain of time. The results are received in an app and stored for tracking purposes. After ensuring security and legal aspects regarding anonymization of personal data, these data can be shared with medical authorities to help control the spread of the disease and prevent future outbreaks, as well as to prescribe the right treatment. After taking the test, the user should act accordingly to the results, whether this means having to take precautionary measures or resuming daily life. In terms of the customer, being able to take a fast test supposes gaining freedom for visiting patients of risk or for traveling, for example when the government requires it (see Figure S3 for a detailed study of the needs from a customer perspective and our value proposition). Additionally, our device is also adaptable and multiplexable, thus offering high scalability. With the same assay, we can detect several different biomarkers, so it can be adapted to different illnesses, such as SARS-CoV-2, HIV, dengue, or even cardiac markers [15]. What should only be adjusted in this case consists of the corresponding antibodies of the cartridge. Therefore, we are building a platform that can offer a wide range of possibilities. We envision not only that our product can be a key element in the future, but that it can also fill the gap in the market today.

5.4 Business feasibility

In this section, we present a business feasibility study, where we describe the required resources as well as a strategy to commercialize our product. In terms of the structure of the organization, the SenSwiss startup will consist of the core team supported by technical advisors. The core team will start with 8 members, out of which there is a CEO, a CFO and six engineers who will also take care of quality and regulatory aspects. Researchers from the Bionanophotonic Systems Laboratory at EPFL will give expertise and counseling for the bioassay and the technology, and the Advanced MicroFluidics (AMF) company for the microfluidics and automatization of the assay. Additionally, we will benefit from the EPFL Tech Launchpad incubator, which will help us to launch our startup, as well as hire recent graduates to expand the team.

We have defined a timeline that consists of the following upgrading stages. First of all, a set of investors including venture capitalists (such as TBG AG, Abingworth or Healthcare Angels) and grant funding (e.g. XGrant, Innogrant) need to be found in order to continue the development of the device. This includes automatization of the sample preparation, cleaning and waste management processes, further optimization of the detection of the intact virus, optimization of the cartridge for multi-sample detection and development of the mobile application for results tracking. The phase of R&D will be done in the first two years, as well as the obtention of the CE certification. After that, the product will be launched to the swiss market in 2024, in collaboration with the Swiss government by being part of their influenza prevention campaign. We will install a first number of 30 to 50 ATMs in 4 major cities in Switzerland: Zurich, Geneva, Lausanne and Basel[16]. This will be our pilot series. A further increase in the number of testing stations installed in Switzerland will be done the following year, as well as the start of the implementation of our device in indoor spaces, such as shopping malls, pharmacies, kiosks and supermarkets. These will be benefited from the income of the renting of the ATM location, as well as the inflow of new customers. By 2028, we will have expanded to 7 countries in Europe and after that, we will enter the global market by selling to Japan and the United States.

Regarding the suppliers, we count on establishing key partnerships with providers of hardware, nanohole wafer and biological reagents. The manufacturing and quality control of the device will be done by our company and the assembly of the ATM will be outsourced to a manufacturing expert company. In further stages of our development, manufacturing of the device will also be outsourced to them. The installation and maintenance of the ATMs will be done by trained technicians, who will be determined for each country. Additionally, we will establish a partnership and collaboration with the governments of each country, who will promote the use of

our product and benefit of controlling and monitoring the disease to improve public health during the influenza campaign. By this, we tackle the externality problem, since the individuals can be reassured of their current condition in a simple and fast way. Furthermore, in outbreaks or pandemic situations, they will have an additional motivation to take the test in order to be able to travel, avoid quarantine or attend mass events, according to the restrictions of each government. As mentioned before, the commercialization strategy also includes installing the ATM in indoor places. For this, we will establish a collaboration with shopping mall chains, pharmacy networks such as the Galenica Group in Switzerland and Phoenix group in Europe and kiosks, among others. As an assistance for the commercialization strategy in order to maximize product launches, we will contact Boston Consulting Group (BCG) who are experts in helping companies related to the health sector to bring their product to the market, support pricing strategies and develop sales and marketing initiatives. Another consulting company, CertValue, will be contracted to guide the process of obtaining the CE certification.

5.5 Financial viability

Our business model is based on the feedback from professionals of the healthcare sector, such as researchers, doctors and pharmacists, as well as the general population, thanks to a survey we conducted. We performed a study of costs and revenues for our model, the gain of which is based on the sales of the tests. SenSwiss will be based in Switzerland, where the office and R&D department will be placed, with a future increase in the number of offices in several countries. The cartridges have an estimated cost of 3.5$, which can be reduced through mass production. The cost of taking a test for a user will be 20$, which could be reimbursed by the insurance company, according to the healthcare sector management of each country. This price is competitive with the existing solutions such as current antigenic tests for COVID-19.

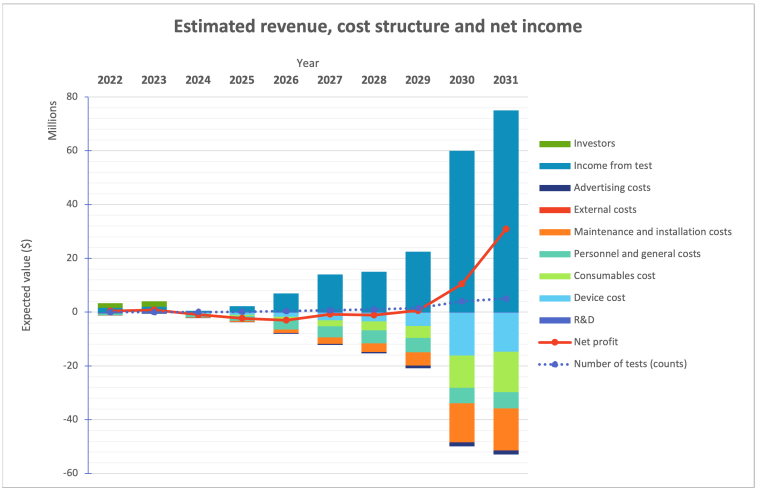


Figure 8: Estimated revenue, cost structure and net income for 10 initial years graph. Number of expected tests performed represented in dashed line (counts).

We consider two different scenarios for the calculation of our gross profit, which consist of the regular seasonal outbreaks of influenza and an epidemics situation. Therefore, we expect a profit of 20'000$ per 1000 tests based on reaching around 10% of the yearly medical consultations done on influenza in ordinary conditions once launched in the market. We expect around 500 tests per year per machine at the pilot series, and an increase to 1000 per year per machine when expanding to Europe and worldwide. At the end of our third year in the european market, we aim at having at least 1000 ATMs placed around the major capitals and big cities. At the end of our prediction, in 2031, our goal is to have at least 5000 ATMs placed worldwide.

We estimate two years for R&D and CE certification, necessary for the commercialization of the device, totalizing around 2'800'000$ cost, which comprises human resources, consumables for the tests, development of the machine, rental of spaces, taxes, consulting, advertisement campaign and regulatory aspects. The exact calculations are described in Table S4. We hope to rise 3'500'000$ from venture capitalists and grants to finance our development. With these estimations, our break-even point will occur in the first year of our expansion to the world (see Figure S4). With this, in 10 years, the initial seed will be multiplied by ten, reaching a net income of 30M$. In the case of an epidemic, the profit will depend on the year of the outbreak in relation to the stage of our development (Figure S6). We hope the market share will rise up to 30% of the testing technologies that are in the market if the outbreak is in 10 years from now.

6 TEAM AND SUPPORT

6.1 Contributions of the Team Members

- **William Verstreaten (Team Captain):** William was the main player of the biology development of the sensor, choosing the right antibodies and developing the procedure to deal with the saliva matrix. He also helped with the coding of our readout system.
- **Deborah Scherrer Ma (Team Captain):** Deborah was the main person to take care of administrative duties and directing the team to a common goal. She was also part of the business team, where she helped in the ideation of the business model and the contact with partners and advisors. She also took part in the development of the bioassay.
- **Blanche Berneron:** Blanche was part of the business team participating in contacting people, companies and administrative matters and developing the business model. Also, she directed the promotion aspects and was part of the biology team, testing the chips and optimizing the protocols.
- **Janet van der Graaf Mas:** Janet was part of the business and biology team. She contacted researchers from Switzerland and Catalonia to give us insights into the current status of POC devices for virus detection, participated in the development of our business model and studied and prepared the biological material for the molecular recognition of our assay.
- **Théo Mayer:** Théo was the leader of the cartridge team, responsible for developing the fluidics approach for our device, as well as its prototype assembly. He also studied the feasibility of a thermal contrast amplification reader for LFA.
- **Sara Chehtite:** Sara was part of the cartridge team and helped develop the cartridge as well as the prototype's design. Additionally, she did an initial study of the technology ELISA-on-chip with magnetic beads.
- **Aviv Huttner:** Aviv was part of the cartridge and prototyping team. She helped develop the cartridge design and worked on the overall design of the prototype. She also helped with the coding on the Raspberry pi. She also worked on the simplified digital ELISA technology, in the end not used.
- **Eloi Schlegel:** Eloi was our promotion guy. He was in charge of the promotion of the team on Instagram, LinkedIn and Facebook. He worked on the instagram take-over and the communication in general. In his free time, he took care of the mental health of the team. :)
- **Ju Wu:** Ju Wu helped with coding and setting up the Raspberry pi.
- **Alina Mingchi Hou:** Alina was a member of the business team, helping with contacting people and gathering information. She also participated in the coding and connection of the computer with the readout system.
- **Nikhil Mahtani:** Nick was part of the business team, helping us in contacting researchers and developing our business model. He also played a great role in taking the minutes and organizing our meetings.
- **Farida Elharouni:** Farida was part of the business team. She contacted companies for consultation and for seeking feedback while developing the business model. She also assisted the cartridge team with problem solving and design.
- **Odysseas Chaliotis:** Odysseas gave support in literature research and new ideas, especially for dealing with saliva.
- **Kamalesh Kumar:** Kamalesh participated in the business team.

6.2 People who have given support

Support was given mainly by our supervisors, **Dr. Philippe Renaud** and **Dr. Arnaud Bertsch**, with whom we had frequent meetings to discuss our progress and problems. **Alexandre Daniker**, who was part of the EPFL SensUs team in 2019, provided us with useful guidance based on his previous experience. Additionally, **Dr. Alexander Belushkin** helped us by introducing us to the technology and letting us use his own EOT-setup

for early testing. Additionally, we received advice and coaching from Professors Sandrine Gerber and Sebastian Maerkl, Dr Marc Friedli, Dr Josep Lluís Bedini, the companies TPP and Novartis, CHUV Hospital and several local pharmacies and insurance companies.

6.3 Sponsors

For the development of our biosensors, we had three sponsors:

- **Thorlabs:** provided us with the hardware of the device.
- **AMF Medical:** helped in the design of the microfluidics of the device and provided us with one of their pumps.
- **EPFL:** gave us the structure for developing our sensors, including labs, a meeting room and most of the materials needed.

7 FINAL REMARKS

Currently, our prototype allows a user-friendly interaction with people in the healthcare field as pharmacists, technicians and doctors. Nevertheless, as we aim at making our device usable to anyone, automatization processes must be implemented. There are three main modifications which need to be made. First, as mentioned before, the current graphical user interface requires the user input to select the region containing the capture antibodies. This process can be skipped by implementing a machine learning code that will allow the automatic detection of the area of interest. Secondly, we would like to automatize the sample handling so that the only input from the user is to provide a saliva sample inside of a small vial to the device. Furthermore, the flow control will be automatized by using a microfluidic pump and an autofocus system must be added. Thirdly, a mobile application could be developed in order to receive the results in an easy manner as well as to keep tracking of the positive cases and allow easy contact with doctors.
We are currently working on obtaining further data with our setup, in order to demonstrate the feasibility of the technology for this use and present it.

The development of our prototype would not have been possible without all the people who supported us, our supervisors, advisors, our university and the hard work of our team captains and team members. We want to express our gratitude to the SensUs organization for making this competition possible. We are really looking forward for the SensUs Innovation Days to share everything we learned with you and, most of all, have fun.

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9 APPENDIX

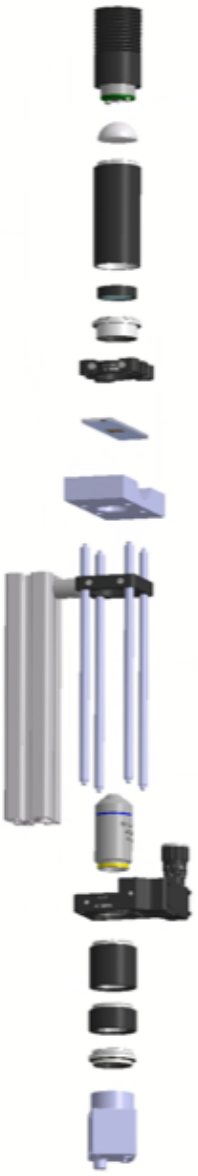

Figure S1: Detailed device setup.

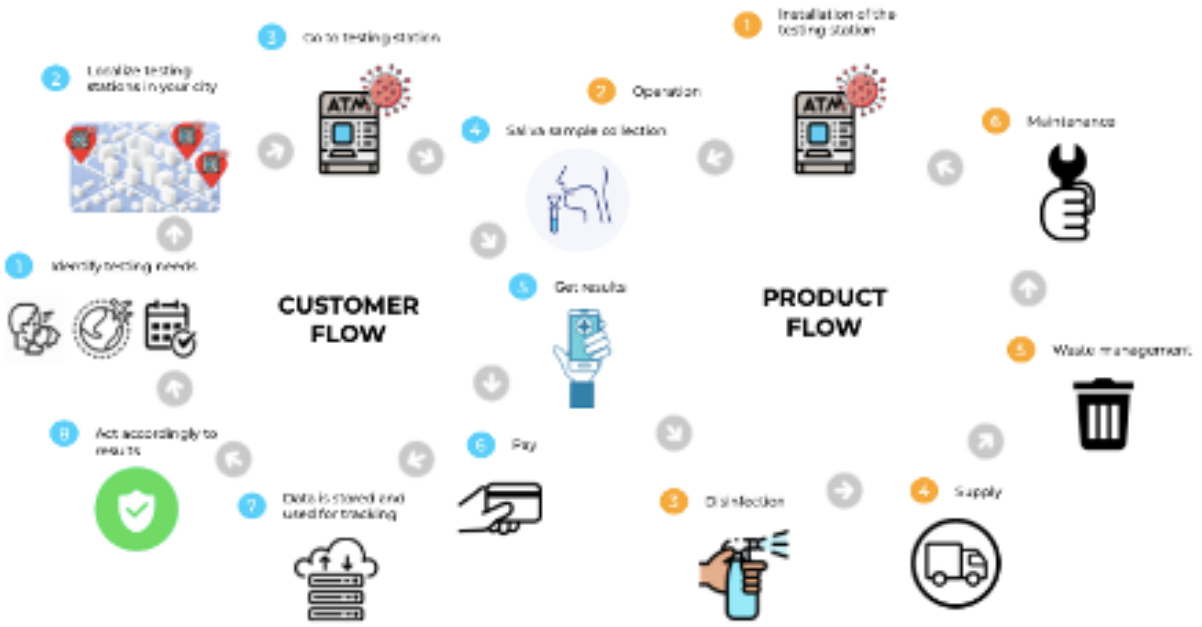

Figure S2: Customer and product flow for the ATM structure.

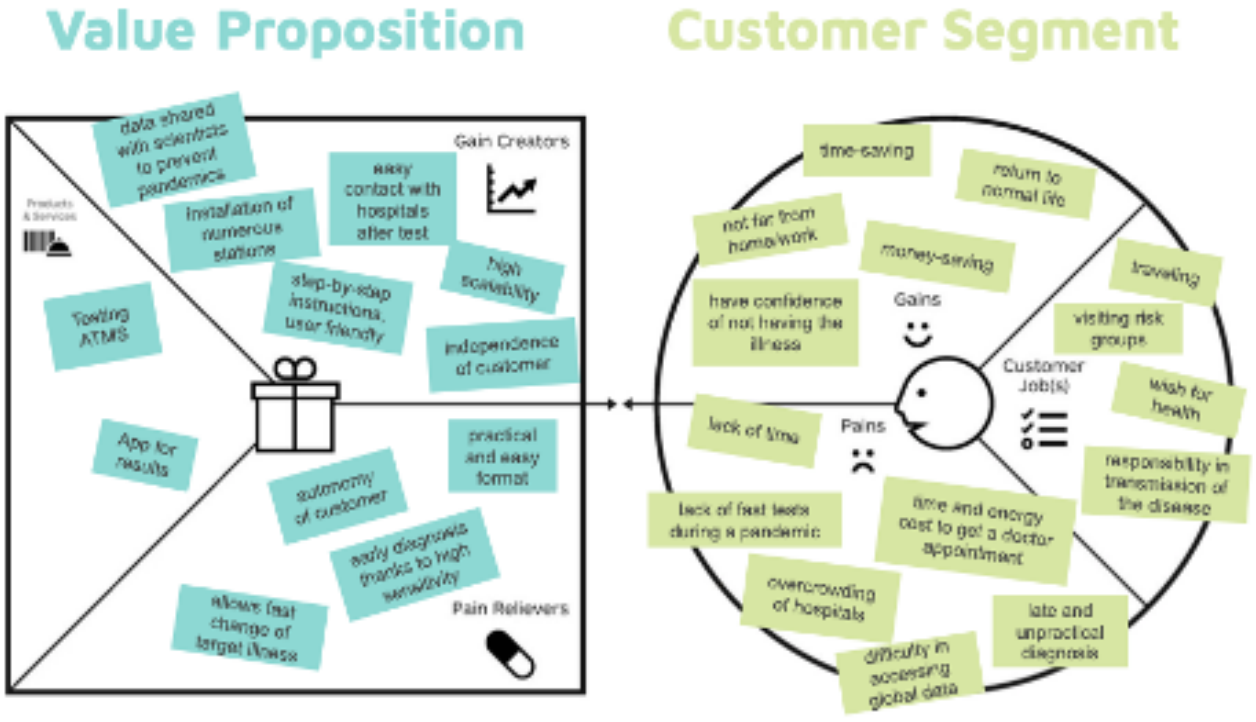

Figure S3: Value proposition and customer segment analysis for the proposed business model

		R&D phase		Switzerland		Expansion to Europe			Expansion to the world		
	Number of stations			50	150	350	700	1000	1500	4000	5000
	Year	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031
Cash outflows											
R&D		500 000	500 000	250 000	250 000	250 000	250 000	250 000	250 000	250 000	250 000
R&D epidemics		1 000 000	1 000 000	1 000 000	1 000 000	1 000 000	1 000 000	1 000 000	1 000 000	1 000 000	1 000 000
Human resources		480 000	720 000	1 020 000	1 512 000	3 264 000	3 744 000	4 512 000	4 896 000	5 202 000	5 406 000
Biosensor Device + ATM		12 000	12 000	250 000	450 000	600 000	1 050 000	810 000	1 250 000	6 250 000	2 500 000
Consumables (total)		5 000	5 000	87 626	393 876	1 225 126	2 275 117	3 250 117	4 500 108	12 000 108	15 000 108
	Cost per test	5	5	4	4	4	3	3	3	3	3
	# tests	1000	1000	25036	112536	350036	700036	1000036	1500036	4000036	5000036
Consumables in case of epidemic (total)		5 000	5 000	875 000	2 625 000	6 125 000	11 375 000	16 250 000	22 500 000	60 000 000	75 000 000
	Cost per test	5	5	4	4	4	3	3	3	3	3
	# tests in case of epidemic	1 000	1 000	250 000	750 000	1 750 000	3 500 000	5 000 000	7 500 000	20 000 000	25 000 000
Maintenance				72 000	324 000	1 008 000	2 016 000	2 880 000	4 320 000	11 520 000	14 400 000
Maintenance (epidemics)				144 000	432 000	1 008 000	2 016 000	2 880 000	4 320 000	11 520 000	14 400 000
Installation				60 000	120 000	240 000	420 000	360 000	600 000	3 000 000	1 200 000
Renting machine space				120 000	360 000	840 000	1 680 000	2 400 000	3 600 000	9 600 000	12 000 000
Advertising			50 000	50 000	50 000	300 000	400 000	500 000	1 000 000	1 500 000	1 500 000
External fees											
	Advisors			10 920	10 920	12 480	12 480	12 480	12 480	12 480	12 480
	Consultancy	5 000	10 000		5 000			10 000			
	Audit		6 000		3 000		3 000		3 000		3 000
Regulatory			15 300	10 200	1 000	1 000	1 000	1 000	15 000	15 000	1 000
Expenses											
	Rent and costs	14 400	14 400	28 800	55 200	110 400	172 800	230 400	345 600	460 800	576 000
	Insurance	197 403	197 403	197 403	197 403	197 403	197 403	197 403	197 403	197 403	197 403
Taxes			63 930	124 825	0	0	0	0	0	0	1 408 725
Taxes (epidemics)			0	0	169 466	1 370 817	4 360 452	10 895 632	19 886 795	33 442 853	73 612 773
	Total (regular conditions)	$1 213 803	$1 594 033	$2 281 774	$3 732 399	$8 048 409	$12 221 800	$15 413 400	$20 989 591	$50 007 791	$54 454 716
	Total(epidemics)	$1 713 803	$2 030 103	$3 766 323	$6 990 989	$15 069 100	$26 432 135	$40 058 915	$59 626 278	$132 200 536	$187 408 656
Cash inflows											
Investors											
	Venture capitalists	1 500 000	2 000 000								
	Grant fundings	140 000									
Revenues		0	0	500 720	2 250 720	7 000 720	14 000 720	15 000 540	22 500 540	60 000 540	75 000 540
	Price per test			20	20	20	20	15	15	15	15
	# Tests			25 036	112 536	350 036	700 036	1 000 036	1 500 036	4 000 036	5 000 036
	Selling										
Revenues (in case of epidemic)		0	0	5 000 000	15 000 000	35 000 000	70 000 000	100 000 000	150 000 000	400 000 000	500 000 000
	Price per test			20	20	20	20	20	20	20	20
	# Tests			250 000	750 000	1 750 000	3 500 000	5 000 000	7 500 000	20 000 000	25 000 000
	Selling			0	0	0	0	0	0	0	0
	~~Renting~~										
	Total (regular conditions)	$1 640 000	$2 000 000	$500 720	$2 250 720	$7 000 720	$14 000 720	$15 000 540	$22 500 540	$60 000 540	$75 000 540
	Total (epidemics)	$1 640 000	$2 000 000	$5 000 000	$15 000 000	$35 000 000	$70 000 000	$100 000 000	$150 000 000	$400 000 000	$500 000 000
Net cash flow											
	Total (regular conditions)	**$426 197**	**$832 164**	**($948 889)**	**($2 430 568)**	**($3 478 257)**	**($1 699 337)**	**($2 112 197)**	**($601 248)**	**$9 391 501**	**$29 937 325**
	Total (epidemics)	**($73 803)**	**($103 906)**	**$1 129 771**	**$9 138 782**	**$29 069 682**	**$72 637 547**	**$132 578 632**	**$222 952 354**	**$490 751 818**	**$803 343 162**

Figure S4: Financial expectations with cash cost structure, estimated revenue and net income. In yellow, expected values for an epidemics situation, based on COVID-19 data[17, 18, 19, 20, 9]

Team members											
		R&D phase		Switzerland		Expansion to Europe			Expansion to the world		
		2022	2023	2024	2025	2026	2027	2028	2029	2030	2031
Director/strategic											
	CEO	1	1	1	1	1	1	1	1	1	1
	CFO	1	1	1	1	1	1	1	1	1	1
	Project Manager	0	0	1	1	1	1	2	2	2	2
Engineer											
	Biological	1	1	1	1	2	2	2	2	2	2
	Mechanical	1	1	1	3	2	2	2	2	2	2
	Fluidics	1	1	1	2	1	1	1	1	1	1
	Hardware code	1	1	1	1	1	1	1	1	1	1
	App Developer	1	1	1	1	1	1	1	1	1	1
	Quality	0	0	1	1	1	2	2	2	2	2
Administration											
	Comptable	0	1	1	1	1	1	1	1	1	1
	RH	0	1	1	1	2	2	2	2	2	2
Sales											
	Directeur Comercial	0	1	1	1	1	1	1	2	2	2
	Marketing	0	1	1	1	2	2	2	2	2	2
	Digital	0	0	1	1	1	1	1	2	2	2
	Field Sales person	0	0	1	2	10	12	15	15	15	15
Regulatory											
	Regulatory engineer	1	1	1	1	1	2	2	2	2	2
	Field engineer	0	0	1	1	5	6	10	12	12	14
Total		8	12	17	21	34	39	47	51	51	53

Figure S5: Human resources of the company for the first ten years of the development.

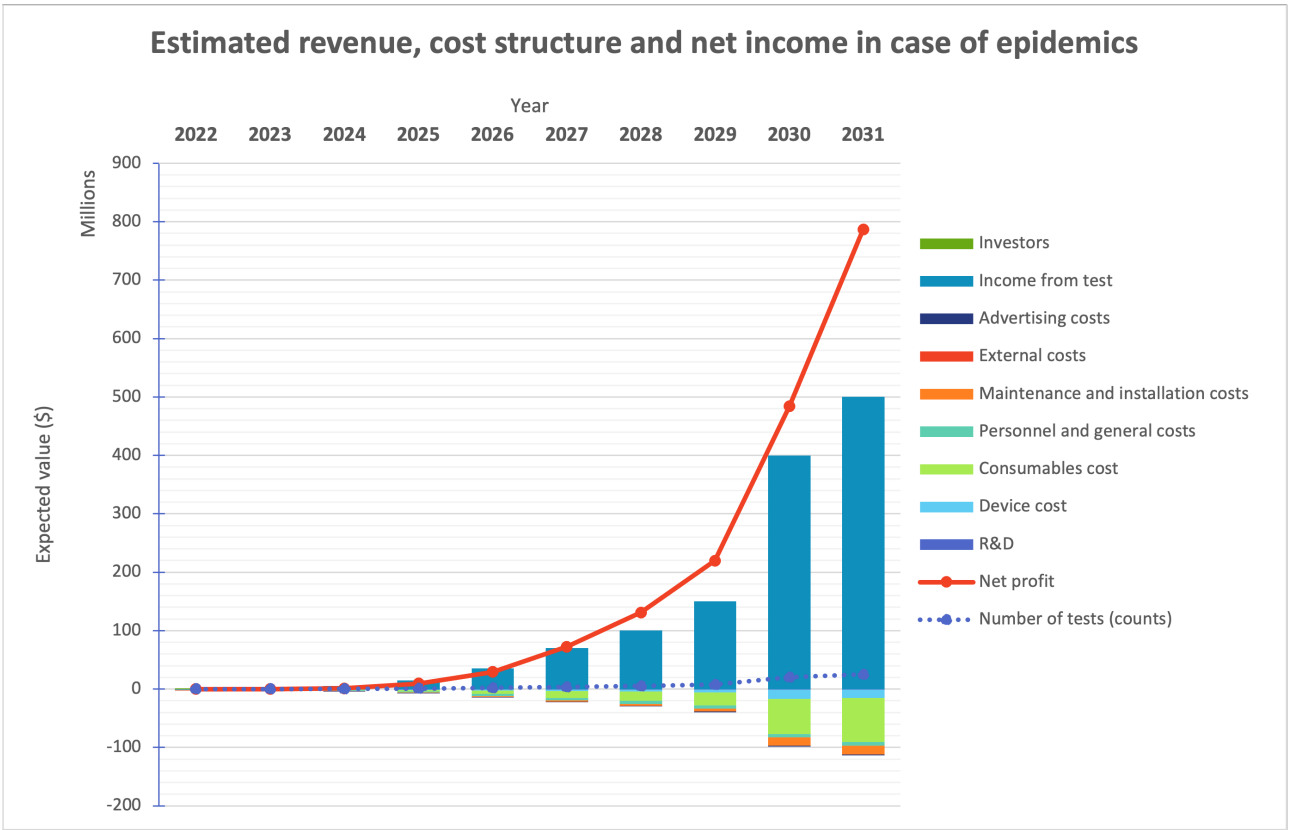

Figure S6: Estimated revenue, cost structure and net income graph in case of a pandemic. Number of expected tests performed represented in dashed line (counts)