# Team Results Document


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# 1 Abstract

In order to tackle a current major health issue and cause of death, Sepsis, our team at Lau'Sens developed a novel biosensor allowing for quantification of the acute infection biomarker interleukin 6 (IL-6) in human plasma. Based on the Extraordinary Optical Transmission (EOT) phenomenon, our sensor measures the concentration of IL-6 in a sample using a nanohole array functionalized with (anti-)IL-6 antibodies in combination with functionalized gold nanoparticles. Thanks to the skillful combination of our cartridge with a micro-fluidics and an innovative autofocusing system, we can correlate nanoparticle binding events with IL-6 concentrations both rapidly and accurately.
Neonatal sepsis alone is responsible for over 13% of neonatal deaths world-wide while being especially deadly in low- and middle-income countries. Missing reliable and fast diagnostic methods often push doctors and healthcare providers to start antibiotics treatment before being able to confirm their sepsis suspicion. On top of compromising the health of the neonates, this contributes to the concerning global increase in antibiotic resistance. The use of our sensor will reduce avoidable antibiotics treatments while being cost-effective and user friendly. Driven by a desire to make change happen, we believe our sensor to be an upcoming game changer in the fight against Sepsis.

# 2 Biosensor system and assay

## 2.1 Molecular recognition and assay reagents

The bioassay used in our sensor relies on a protein-based sandwich assay similar to an ELISA, that uses two different antibodies: a capture and a detection antibody, both binding to two distinct epitopes of our target ligand, here interleukin-6 (IL-6). Both antibodies are monoclonal, to ensure higher specificity, binding only to the corresponding epitope of the IL-6 protein we want to detect. First, gold nanohole array (Au-NHA) surfaces are uniformly functionalized with copoly-DMA-MAPS-NAS polymer (MCP-2), containing activated amine reactive NHS-ester groups. This ensures stable and reproducible antibody immobilization, which is of importance in the case of a flow addition onto the surface. Then, capture antibodies (CP30611 abcam, [10]) are spotted using cellenOne X1 piezoelectric noncontact microdispenser (Cellenion) at precise locations on the chip surfaces (Au-NHA surfaces) [2], and adhere to it chemically via the activated NHS-ester groups that enable covalent immobilization of molecules through amino groups (Fig. 1 A). The size of the spots varies from 150 to 170 $\mu$m. The polymer MCP-2 helps prevent non-specific gold nanoparticle (Au-NP) binding on the off-spot regions, and fouling by plasma proteins [14]. Afterwards, the surface is passivated with SuperBlock™ Blocking Buffer (ThermoFisher Scientific) for 2 minutes to avoid nonspecific binding of molecules to the non-spotted parts of the surface. Thereafter, the plasma sample is treated with 25 mg/mL heparin in phosphate buffer saline (PBS) 1X (lithium salt) to avoid aggregation of the Au-NPs. The sample is then mixed with the reaction buffer composed of PBS 1X, NaOH (50mM) and Tween20 (2.5%). NaOH increases the pH of the sample to 8.5, ensuring the best interaction between the antibodies and the analyte [20, 15]. Tween20 is a surfactant added to the buffer to limit the non-specific interactions between Au-NPs. Finally, the 100 nm diameter Au-NPs coated with 10 kDa PEG and activated with EDC-NHS are functionalized with the detection antibody (CP35549 abcam, [10]) and mixed into the diluted sample which is then flowed over the gold surface (Fig. 1 B). The detection antibodies will bind to the IL-6 molecules which will also bind the capture antibodies at the surface. Upon shining light through the Au-NHA surface, the light intensity is locally decreased where binding of the Au-NPs to the ligand occur. This allows to detect binding events and further estimate the target concentration (Fig. 1 C).

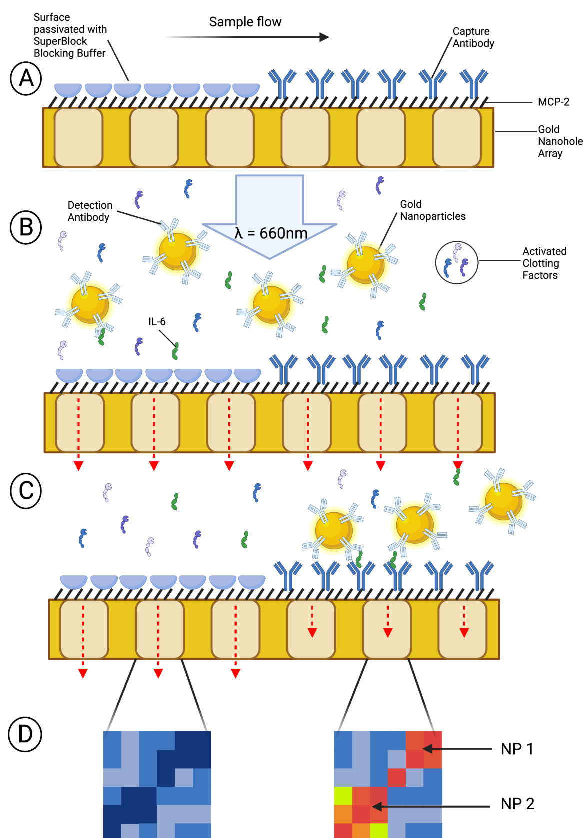



**Figure 1:** Scheme of the molecular recognition sandwich assay. A: Representation of the gold nanohole array (Au-NHA) after being functionalized with MCP-2 then spotted with the capture antibodies and passivated with SuperBlock™. B: The plasma sample treated with heparin and mixed with the reaction buffer is added to the gold surface. C: In the presence of IL-6, the functionalized gold nanoparticles (Au-NPs) bind to the capture antibodies enabling the dectection of IL-6. D: Measuring the pixel ratio of the captured image, we obtain the concentration of IL-6 in the sample. (red spot = intensity dips)[1]

## 2.2 Physical Transduction

To enable the detection of IL-6 molecules in plasma, we use an optical transduction mechanism known as: "nanoparticle-enhanced plasmonic Au-NHA sensing". This technique is exploiting the extraordinary optical transmission (EOT) of NHAs and the optical properties of Au-NPs. Au-NHAs are arrays of holes having a sub-wavelength diameter on a metallic surface. They are known to exhibit EOT, where Au-NPs disturb the near-fields of the Au-NHAs locally when binding to the target analyte, creating a strong

local transmission suppression in the far-field [16]. These distortions in the transmission from nanoholes' vicinity can be detected under narrow-band illumination at the EOT peak in the visible range. This allows to create plasmonic intensity heatmaps to visualize individual Au-NPs as spots with high contrast (Fig 1 D) [1, 2]. The latter can be observed using a simple CMOS camera to easily detect single-binding-events of Au-NP at the surface.

## 2.3 Cartridge technology and microfluidics

The Au-NHAs are made of silicon oxide covered with a titanium adhesion layer and a gold layer using deep ultraviolet lithography (DUVL) and ion beam etching (IBE) for low-cost wafer-scale fabrication [13]. The holes have dimensions of 200nm in diameter with a periodicity of 600nm.

The Au-NHA chip is placed on a polydimethylsiloxane (PDMS) support with a square slot, with the microfluidics cartridge placed on top (Fig. 2). The whole system is clamped by a 3D printed element, which occupies a volume of 50x50x20mm (Fig. 3). The microfluidic cartridge is composed of 2 PDMS layers, fabricated in the cleanroom. The bottom layer contains channels above the surface of the Au-NHA chip, while the top allows easy insertion of fluids by extending the channels to the sides. Three channels with cross-sections of 50x500 $\mu$m are formed by pouring a uniform layer of PDMS on a silicon mold that is patterned by photolithography. After degassing and curing, the chips are cut, and then bound by plasma bonding. Inlets and outlets are made with a circular puncher. The dimensions of the cartridge are 30x20x9mm; however, the height can vary slightly due to the variability in the pouring process. The 3D printed part allows us to re-use the microfluidics cartridge and to easily replace the Au-NHA chip. The whole cartridge is placed on the moving platform of the reader. Samples are inserted into the cartridge by SPM Microfluidics Pump supplied by Advanced Microfluidics (AMF) to generate a flow through the channel and to overcome the mass transport limited regime. Only one channel is used to detect IL-6, the other two are to integrate other biomarkers in the future (see Section 3). Water and detergent are used to wash the tubes to avoid cross contamination between each measurement.

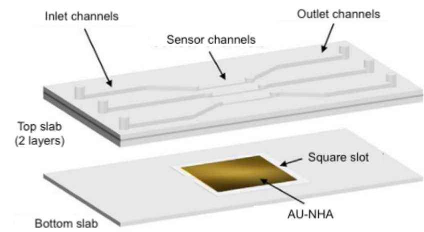



**Figure 2:** Composition of the microfluidics cartridge [22]

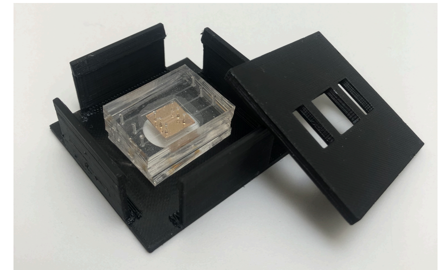


**Figure 3:** 3D printed support with microfluidics cartridge

## 2.4 Reader instrument and Graphical User Interface (GUI)

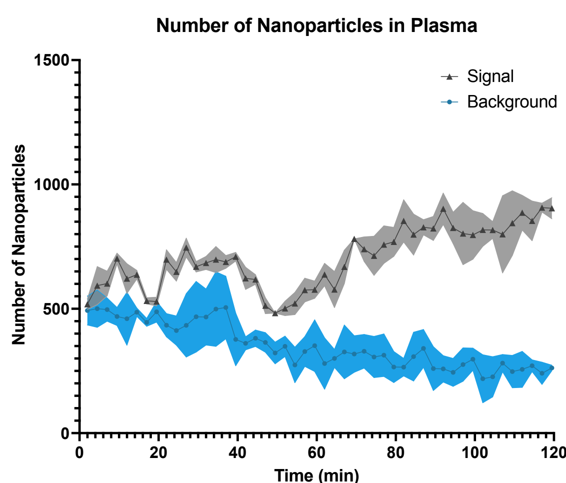
To finally translate the optical signal into an IL-6 concentration, we use a 3D-printable bright field microscope. The cartridge, the pump and the optical system are incorporated in a structure, with a compact design which satisfies the constraints of the competition (18x16x45cm) (Fig. 7). A motorized moving stage based on the OpenFlexure design [21] allows us to navigate the surface of the chip and find the best position for focusing. The user simply needs to place the cartridge onto this moving stage where it will be clamped. We have also developed a graphical interface that allows the user to move the stage and initiate the autofocusing. Based on the work of Bowman et al. [11], we use Laplace variance as our sharpness metric to detect when the image of our sample is optimally focused on the camera. This allows us to avoid user-to-user variability in this process, which requires extreme precision to obtain robust results, thus making our sensor remarkably user-friendly. The overall reader design is cheap and highly customizable, allowing for a low-cost reader with outstanding performance. The motion precision is submicrometric, and the illumination has an adjustment system that allows for Köhler illumination, which results in improved contrast and a reduction of artifacts. An overview of the hardware and user interface is presented in Figure 7 and Figure 8. After images of the chip surface have been acquired, the user will be asked to select the regions of interest corresponding to the nanospots on the GUI. The images are then processed based on morphological operations to automatically and reliably compute the concentration of IL-6 from the auto-focused images, which will then be displayed to the user on the interface.

# 3 Technological feasibility

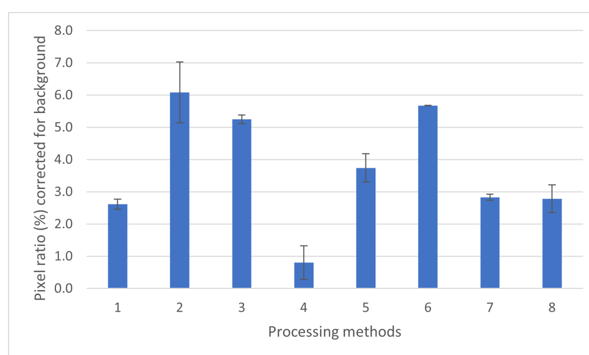
## 3.1 Molecular recognition

To ensure the optimal molecular recognition of our system, we tested the effect of the plasma matrix on the protein-antibody interaction. We also optimized the final antibody pair ensuring the best possible signal. We first used a typical ELISA sandwich antibody pair of capture and detection anti-IL-6 to test the baseline signal of the interaction. Afterwards, as it would have been time-consuming to test the five recommended pairs sent by HyTest with our system, we opted for the biolayer interferometry technique (BLI). BLI is a label-free biosensing technology that analyzes biomolecular interactions in real-time. It measures the interference pattern of white light that is reflected from a layer of biomolecules immobilized on the surface of a sensor tip in solution [3]. The BLI allowed us to select the best antibody pair (L519-L395) as shown in Fig. 9. In the future weeks, we will chose the final antibody pair used in our system by selecting the best pair between the best antibody pair from the BLI results and the initial pair we used to test our system. It will be the one giving the best signal-to-noise ratio with our final prototype.

To find the best protocol for our bioassay and adapt it to the plasma matrix, it was necessary to increase the signal-to-noise ratio and decrease the non-specific binding in this complex sample compared to PBS. For that purpose, we used an EOT-setup similar to our final prototype. We tested different protocols to reduce the noise induced by the high viscosity of plasma but also the presence of many active clotting factors. These protocols included, but were not limited to: heating, centrifuging, increasing the surfactant concentration, treating with heparin lithium salt and also changing the blocking buffer. We decided to use the SuperBlock™ Blocking Buffer, which gave us better results than the pierce milk blocking buffer [23]. The combination showing the best results was selected as proposed in the Section 2 and can be seen in Fig. 4b. Using this EOT-setup combined with the sample preparation, we were able to detect IL-6 in plasma at a concentration of 1 ng/ml (as shown in Fig. 4a), by acquiring and processing images of our chip in real-time. From these results, the cutoff time i.e., the moment from when the signal was significantly different from the background with our static system was around 1 hour, as can be seen on Fig. 4a. 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 cutoff time with the microfluidics system integration, as well as the LOD of our setup, which we expect to be around 50 pg/mL, based on previous experiments performed by Alexander Belushkin [1, 2].




**(a)** Live measurements of IL-6 1 ng/mL in processed plasma sample. Evolution of the number of NPs in PLasma with time.




**(b)** Signal corrected for background for different types of sample preparations: Horizontal axis: 1. PBS with Tween20 (2.5%), [NP] 0.13 nM and Pierce clear Milk as Blocking buffer. 2. PBS with Tween20 (2.5% ), [NP] 0.13 nM and SuperBlock™. 3. Plasma sample with heparin (25 mg/mL), Tween20 (2.5% ), [NP] 0.13 nM and SuperBlock™. 4: Plasma sample with heparin (25 mg/mL), Tween20 (2.5% ), [NP] 0.26 nM and SuperBlock™. 5. Plasma sample with heparin (25 mg/mL), Tween20 (5%), [NP] 0.13 nM and SuperBlock™. 6. Same conditions as the third experiment with a heating incubation at 35°C. 7. Same conditions as the third experiment with sample spinning (1300 rcf, 10 min., 15°C). 8. Plasma sample without heparin treatment, Tween20 (2.5% ), [NP] 0.13 nM and SuperBlock™. Notice that the first 2 experiments were done in PBS buffer.

**Figure 4:** Results demonstrating the technological feasibility of the bioassay

## 3.2 Fluidic cartridge

A channel height of 50 µm minimizes the incubation time since it reduces the average diffusion distance between the molecules in the channel and the Au-NHA, while being easily fabricated without stiction problems. The flow in the channel induced by the pump is simulated on COMSOL multiphysics (Finite Element Method (FEM) software) to optimize it. On one hand, the assay is in mass-transport limited regime if the flow is slow 5 and on the other hand the captured Au-NP yield from a finite volume is lower if the flow is faster. Initially, only one channel is used to detect IL-6 and the other two can be used if the first one is blocked. In the future, these channels will allow multiplexing by dividing the plasma sample in three different regions with specific capture antibodies for each region. PDMS is an easy to mold material for fast prototyping, transparent not to disturb light transmission, shock resistant, bio-compatible, and cheap for prototyping purposes. The latter material is however a limitation of the cartridge if the sensor is produced at a large scale. PMMA would be preferred in this case. The 3D printed support allows the replacement of the Au-NHA to reuse the microfluidics cartridge which is preferable from an environmental standpoint. For recycling, it is also nice to be able to separate the different materials. However, it adds an assembly step for each measurement which is less user-friendly than a pre-made cartridge which can be put directly inside the sensor and thrown away after use. The pump induces a flow in the channel to improve kinetic assay but it also allows to clean the cartridges in an automated way.

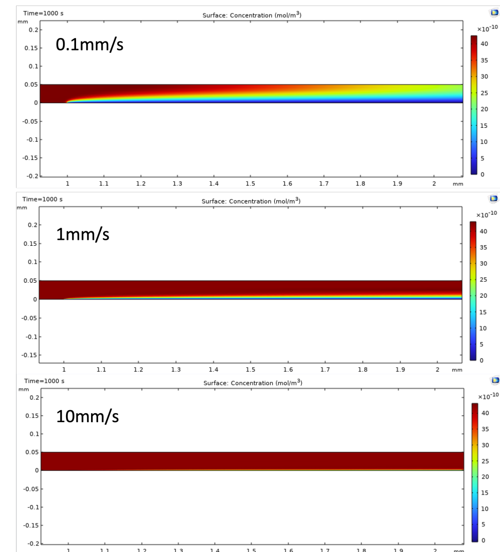



**Figure 5:** Simulation of the flow by representing the protein concentration along the channel: 0.1mm/s mass-transport limited, 1mm/s intermediate regime, 10mm/s kinetic limited

## 3.3 Reader instrument and user interaction

The reader we have developed aims to make the process of analyzing patient samples as efficient and simple as possible. We are able to move across the chip with excellent accuracy, the achievable step size in z is $50nm$, while for the x-y directions it is of $88 \pm 6nm$ [21]. A change in the direction of the motors introduces motion uncertainty caused by the backlash of the gearing mechanisms limited to $6.9 \pm 1\mu m$ [21]. The stage is able to move in x-y-z with a maximum travel of 12x12x4mm, respectively, which allows us to completely cover the surface of the chip and be able to focus even starting at completely out-of-focus positions.

We designed an illumination tower to embed the optical system, connected by 3D-modeled components. This allows us to change the lens positions and apertures to achieve the best possible illumination on the sample plane.

Our set-up allowed us to develop a software with a graphical user interface which controls the stepper motors via USB serial. The purpose of the program we developed is to acquire images, evaluate their sharpness metric, and, in an iterative process, control the motors to reach a position that maximizes these values, corresponding to the position where our image is in focus. In addition, it is possible to move the stage in x-y direction to move around the surface targeting regions of interest. This brings us benefits both in terms of efficiency and precision.

The current reader allows us to move and perform autofocus on the chip surface with satisfactory results. Our goal in the coming weeks is to improve the stability and compactness of the hardware and in parallel make the autofocus process more efficient in terms of accuracy by including or comparing the sharpness metric currently used with other methods of sharpness evaluation (e.g. JPEG size).

To further simplify the user interaction with our sensor and improve the image processing software, we plan on implementing a robust automatized region of interest selection. The user would then only have to introduce their sample cartridge into the reader, focus and ask for the concentration to be measured by clicking two buttons.

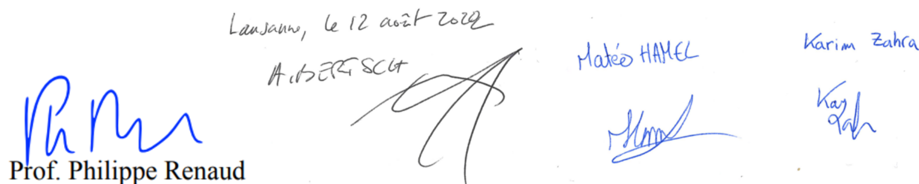
# 4 Originality

## 4.1 Team

Our team developed a biosensor for the quantification of IL-6, combining and optimizing bioassay, microfluidics and reader. After an extensive literature review, we selected and tested three promising methods, namely methods based on electrochemical impedance spectroscopy, slip-chips and extraordinary optical transmission. Recognizing the last method to have the highest potential, we decided to focus on a sandwich immunoassay on a gold nanohole array, as proposed by Belushkin et al. [1]. Indeed, the latter allows for a short time-to-result, which is a critical parameter when targeting the sepsis application. Detection of IL-6 in plasma has already been done previously using sandwich immunoassays [24], such as ELISA, with high accuracy but however long time-to-result [9]. By functionalizing both the array surface and gold nanoparticles, we can detect the presence of the IL-6 cytokine by counting the bound nanoparticles on specific areas of the sensing platform. Although this method is per se not new, our team has adjusted the technology and complied it to the SensUs constraints and requirements through extensive testing. We namely adapted it to a plasma matrix, which we believe to not have been done before. We developed a custom-made PDMS cartridge, including a microfluidics system to embed the gold nanoholes array. With the latter microfluidics system, the detection time decreases considerably. To obtain stable and reproducible results when using microfluidics, the proposed gold array is coated with MCP-2, creating a covalent bond between the capture antibodies and the array surface. To the best of our knowledge, the combination of the MCP-2 coating and microfluidics was not used before for this application. Our reader, which was 3D-printed based on the OpenFlexure Project, includes a custom-made autofocusing system and a user-friendly graphical user interface (GUI). Thanks to the autofocusing system and the microfluidics, our system provides robust results while requiring minimal manipulation and no expertise in the field. Our image processing software has been adapted to the reader and to our sensing platform by the team, combining polynomial background removal with morphological operations. Finally, our innovative business plan is based on the combination of an extensive literature research and interviews with doctors and other health-care professionals, consulting companies, start-ups and regulatory agencies. Selling a service instead of a product aims to facilitate the use of our device in the clinic.

## 4.2 Supervisor

EPFL team developed a biosensor based on Extraordinary Optical Transmission (EOT), a phenomenon first reported by Ebbesen et al. in 1998 [6]. It uses a metal layer in which a nanohole array is patterned with subwavelength spacing inducing an enhanced transmission when irradiated with light at a specific wavelength. When biomolecules are in close proximity to this metal nanohole array, the dielectric conditions change close to that metal layer, inducing a shift in the maximum transmitted wavelength.
Belushkin et al. used this method in 2019 on actual serum samples from patients to detect two sepsis biomarker proteins. The EPFL team adapted this method to plasma, but the main innovation they performed relates to the implementation of different strategies to reduce significantly the assay time. One of them consists in using a functional polymer, MCP-2, to strengthen the bonding between the substrate and the detected biomolecule and consequently retaining the biomolecule of interest for a longer duration in close proximity to the detection surface. Another one is related to the use of dedicated microfluidics to reduce the incubation time required for detection. These two aspects do not relate to the fundamental principle chosen for the biosensor, but on practical aspects required to develop a system that is able to perform the detection of IL-6 in a reasonable time frame, which is a main challenge in the development of a biosensor that can be used not only in a research lab as a proof of concept, but in a "real life" clinical environment.



# 5 Translation potential (max. 3 A4)

## 5.1 Business Model

**PROBLEM**
- A high proportion of neonates wrongly suspected of sepsis (not reliable early diagnosis methods for Neonatal Sepsis (NS))
- When sepsis is suspected, reaction time is critical
- Limited resources in LMICs (overcrowded NICUs, second-hand medical equipment, shortage of healthcare professionals)

**EXISTING ALTERNATIVES**
- Misuse/Overuse of Antibiotic Treatments (ABT)
- Diagnosis based on clinical symptoms
- Laboratory tests (CRP, PCT, manual blood cultures) expensive, not accurate or with long time to result.

**SOLUTION**
- Syndromic test for early sepsis diagnose
- Multiplex solution, giving no false negative results (reduce ABT overuse for neonates)
- Microfluidic cartridge allowing short time-to-result
- User-friendly interface for easy manipulation
- Cost-effective in vitro device

**KEY METRICS**
- 62,5% specificity and 100% sensitivity
- 2/3 of neonates with suspected sepsis prevented from undergoing unnecessary ABT
- 10% of the market size (LMIC's NICU) reached in 15 years

**UNIQUE VALUE PROPOSITION**
- 7'648CHF saved yearly per NICU (156'000 EGP)
- 46 neonate ABTs avoided yearly per NICU (a total of 190'000 in Egypt)
- Product as a service (PaaS) including automatized cartridge supply, maintenance and on-field technical support

**HIGH-LEVEL CONCEPT**
- Antigenic Covid-19 test for NS

**UNFAIR ADVANTAGE**
- Spinoff EPFL (already existing patent, funding, mentoring)
- Favourable Network: NICU Specialists (and other health-care professionals) in Cairo

**CHANNELS**
- Governmental insurance (i.e. Tamin Sehy)
- NGO support (such as Red Cross and Egyptian Neonatal Network)
- International medical exhibitions and conferences, such as InterLab Africa and Africa healthOutsourced sales force
- Word of mouth in medical world

**CUSTOMER SEGMENTS**
- Neonatal Intensive Care Units (NICUs) of public hospitals all over Egypt
- NICU of private hospitals all over Egypt
- NICU all over LMIC's

**EARLY ADOPTERS**
- NICU of Ain Shams public hospital (Cairo, Egypt)
- NICUs of others public hospitals (Cairo, Egypt)

**COST STRUCTURE**
- Fixed costs: regulation, IP rights, R&D, payroll, marketing, infrastructures, consultancy
- Variable costs: cost of goods sold (including cost of insurance and freights, manufacturer margin, ...), agent commissions, IP right EPFL licensing

**REVENUE STREAMS**
- PaaS with the following costs per NICU (with the possibility of monthly payments):
  - 7'900 CHF/year for a 1-year contract
  - 7'655 CHF/year for a 3-year contract
  - 7'409 CHF/year for a 5-year contract

**Figure 6:** Business Model (see Appendix 8.3)

## 5.2 Market description

Neonatal Sepsis (NS) is an urgent global health concern, accounting for 13% of overall neonatal mortality [12]. 99% of the neonatal mortality due to sepsis occurs in Low- and Middle-Income Countries (LMICs) [17], which highlights the huge disparity between High-Income Countries (HICs) and LMICs in NS diagnosis and treatment.

At an early stage, NS is hard to diagnose, as it has aspecific clinical signs that can overlap with other conditions [7]. In LMICs, broad spectrum antibiotics treatment (ABT) is immediately started for all neonates at risk or with clinical signs of sepsis, that is 16.6% of all neonates [17], before their manual blood culture results are available. Time-to-results of manual blood cultures in LMICs is of 7 days, which forces doctors to take action before knowing the result of the neonate's blood culture. Other laboratory tests (CRP and/or PCT detection and complete blood count) can sometimes help neonatologists monitor the effect of different antibiotics and discontinue ABT if possible. However, according to Dr. Yasmin Gamal, Director of the Children's Hospital of the Ain Shams University in Cairo, neonatologists in LMICs mostly rely on their "clinical sense", as these tests are expensive, invasive and often not very reliable. This situation leads to the overuse and misuse of antibiotics in LMICs, contributing to the increase in AB resistance seen as a major threat to global health by the WHO [17, 25].

Please refer to section 5.5 for further precision on the market segment size.

## 5.3 Stakeholder desirability

Early ABT is not without risk for the neonate, as it is notably associated with obesity and asthma [7]. Additionally, as pointed out by Dr. Hossam Amar, Neonatology Consultant of the Ain Shams University in Cairo, early ABT leads to overcrowding of neonatal intensive care units (NICUs) and higher risk of hospital-acquired infections. For the healthcare system, this adds huge financial costs.

In the LMICs market, a frugal innovation, focusing on reducing costs while concentrating on core functionalities as well as optimizing performance level has the strongest product-market fit [8]. We chose NICUs of public hospitals in Egypt as our early adopters. Indeed, Egypt is an ideal beachhead market as it has the $2^{nd}$ highest GDP of Africa and invests a lot of money in healthcare and technology compared to other LMICs [8].

Dr. Gamal described NS as "the nightmare of all neonatologists in Egypt". For the whole Egyptian healthcare system, NS adds huge financial costs. The entire treatment of a neonate cost approximately 12000EGP (600 CHF) per week (see Appendix 8.5.3). In the public sector, all neonates are covered by "Tamin Sehy", the health insurance of the Egyptian government. Therefore, this avoidable ABT adds unnecessary financial burden on the government and families choosing private hospitals to save their newborns.

Compared to our competitors, we propose a cutting-edge biosensor that can detect not only one, but multiple biomarkers with a high sensitivity, rapidly and cost-effectively. Our product detects the concentration of IL-6, nCD64 and CRP. This combination of early-rising biomarkers IL-6 (rising within the first 2-4 hours [7]) and nCD64 (rising within the first 1-6 hours[4]) with a late-rising biomarker CRP (rising within 12-24 hours[7]) enables us to get close to a 100% sensitivity and 62.1% specificity, even at an early stage [5].

Neonates with suspected sepsis will be tested using our IVD device before starting ABT. Thanks to a short time-to-result of less than 15 minutes, medical professionals will be able to prevent unnecessary ABT for 7.5% of all neonates, thereby increasing the bed turnover rate. As we only need to extract a small volume of plasma ($12.5\mu L$) from the neonate's peripheral blood, the latter can easily be collected by capillary sampling from the neonate's heel, as confirmed by Dr. Angela Bikker. This minimally invasive extraction reduces the risk of anemia and potential hospital-acquired infection, as stated by Dr. Basma.

Using our product will save 7648 CHF per NICU per year and prevent 46 neonates ABT per NICU (a total of 190'000 in Egypt), which clearly will benefit the whole healthcare system, starting from neonates and their parents, to NICUs, governmental insurances, medical professionals and non-profit health organizations (see Appendix 8.5.3). Other stakeholders such as the notified body, contract manufacturer and local distribution agency will also benefit from our business (see Section 3).

## 5.4 Business feasibility

In the next two years, with the help of EPFL experts in different laboratories such as the BioNanoPhotonic Systems Laboratory, we will continue conducting the Proof of Concept (PoC) and developing our prototype. Our R&D team will focus on prototyping a new multiplex biosensor able to detect the 3 biomarkers (I-L6, CRP, nCD64), integrating a centrifugal disc to become a point-of-care device and automatizing device monitoring (see Appendix 8.4.1). Additionally, we plan on storing test results in compliance with local regulations. The saved data can then be used to build computational models able to predict neonatal sepsis, whose feasibility and interest have been proven in recent years and could be used as additional indication if ABT should be started immediately [19].

Tests and clinical studies with a bigger population in Cairo's public hospitals will be crucial to validate our biomarker's sensitivity and specificity, as we are currently relying on the values proposed by Dilli et al., and are aware of the limits of such a study [5].

We will focus on licensing, securing capital funds, optimizing our product-market fit, product testing and developing a regulatory plan. We are already in contact with the Technology Acceleration Manager from EPFL's Technology Transfer Office (TTO), Eric Meurville, for guidance and support in developing a viable business model and studying potential exclusive licensing. In order to secure capital and optimize our product-market fit, we will benefit from the EPFL Tech Launchpad funding (Ignition and Innogrant) and expertise (on matters such as designing, prototyping and de-risking). We also plan on contacting venture capitalists and investors (Verve, IFC, IFHA, SIFEM), NGOs and international humanitarian organizations (Red Cross, UNICEF) for support. We already contacted the UN *NGO Liason Unit* to help us reach appropriate NGOs such as the Egyptian Neonatal Network (EGNN).

We plan on developing an optimal regulatory strategy, with the help of our consultants in Switzerland and partners in Egypt, to obtain the CE and free sales certificates in the next two years. We plan for our sensor to get the CE marking via a notified body following the In Vitro Diagnostic Regulation (IVDR) process (EU IVDR 2017/746) [18]. We estimate our sensor to be a class C device, following the IVDR. We are however aware that we need to confirm this classification, as pointed out by Roberto Constantini, founder of Elseemed, a medical device consulting company. The CE marking, combined with a Certificate of Free Sale (CFS), will then allow our product to enter the Egyptian market. To

conform to the market regulations, we have developed a mitigation plan summarizing possible risks (see Appendix 8.4.2). Taking advantage of the multiplexed nature of our sensor, we notably integrated an internal control, thus allowing to correctly handle non-functional tests.

We already contacted our main suppliers to reduce production cost by engaging in long-term partnerships (e.g. ABCAM for antibodies supply) (see Appendix 8.4.3).

As the legal manufacturer, we plan on contacting a contract manufacturer in Egypt having a Quality management system (QMS) and certified ISO 13485. This will allow us to manufacture and assemble in Egypt, avoiding expensive finished product customs and delays.

The distribution, installation and maintenance will be outsourced to an Egyptian agency, Techno Wave. Our future customer service department will ensure customer satisfaction and provide technical training to our agency.

Our commercialization strategy is based on the Product as a Service (PaaS) model. NICUs, subscribed to our product for a recurring fee, will use our biosensor and benefit from our efficient customer support services. Our services will notably avoid the logistics hustle of ordering new chips every 3-4 weeks due to their low expiry duration. Our subscription model will also include automatized device monitoring and maintenance.

The PaaS model has many economical and ecological advantages. We are especially proud to act towards the Sustainable Development Goal 3 of the United Nations, diminishing preventable deaths of newborns. A complete sustainability plan can be found in the Appendix 8.4.4.

Smart marketing will be key to our success. We will participate in international medical exhibitions and conferences, such as InterLab Africa and Africa health, to promote our product and expand our network. We will collaborate with an agency in Egypt to promote and expand our market from the Ain Shams University Hospital in Cairo to all public hospitals in Egypt.

## 5.5 Financial viability

In order to be financially viable, we have to generate sufficient income to meet our operating expenses and allow future growth. As our current prototype is composed of high-performance components such as the microfluidic pump, the CMOS camera and the Au-NH chip, it is not adapted for mass manufacturing. The current pump and camera will be replaced by cost-effective components to find the right balance between price and performance. We estimate that the latter combined with the long-term partnerships with our suppliers will decrease costs by 33%. The production cost of the biosensor and the consumables (Au-NPs and chips) will be of 2862 CHF (see Appendix 8.5.1) and 7.49 CHF respectively (see Appendix 8.5.1). The production rate of the Au-NH chip (currently supplied by the UCSB Nanofabrication Facility) will remain a limiting factor, as it requires the use of a DUV stepper machine, only found in cleanrooms. We will investigate possible alternatives such as producing the chips ourselves to mitigate this problem.

Moreover, mass production comes at a high cost. For instance, our contract manufacturer in Egypt, will have a gross margin of 7%. Importing components into Egypt adds costs, such as cost, insurance and freight (CIF), customs clearance fees, import duties and value-added taxes (VAT). According to Mohamed Khedr, CEO of Techno Wave, the Egyptian government is encouraging local manufacturing and offers financial advantages corresponding to 15% of the Cost of Goods Sold (CoGS).

Distribution, sales and maintenance will be outsourced to our agency which will take 25% revenue commission (see Appendix 8.5.2).

As our commercialization strategy is based on PaaS, we will offer different subscription models, coming with different prices and durations (see Appendix 8.5.3). Based on the cost-plus pricing strategy, the cost for our 1-month subscription fee will be of 659 CHF, without considering extra fees for misuse-related damage to the device or for exceeding a maximum number of consumables. Thanks to our unique value proposition, each NICU can save up to 7'648 per year, as we make a yearly profit of 908 CHF per customer per year (see Appendix 8.5.3).

Our expansion plan begins in Cairo, and we expect to expand to the rest of Egypt in 2026. When we attend 50% of Egypt's market, we will start targeting other countries in sub-Saharan Africa. After reaching 80% of Egypt's market, we expect to reach a plateau, thus the importance of targeting new markets. By the end of 2030, we expect to have reach 4% of Africa's market and later expand to other LMICs (see Appendix 8.5.4).

Raising more than 4 million CHF during the first two years of our PoC to finance our development, a rapid obtention of the CE marking and clinical trials will be key to our success. With the previous estimations, our break-even point will be reached by 2028 when we surpass more than 30% of Egypt's entire market (see Appendix 8.5.2).

# 6 Team and support

## 6.1 Team

**Mehmet Kuzey Aydin and Titouan Marois** were the microfluidics team; they worked on setting up, fabricating and testing the microfluidic system of our sensor. Titouan also did a semester project on adapting a Slip-Chip device for the competition.

**Nerea Carbonell and Camille Pescatore** were part of the software team, focusing on image processing. They also participated in the business team, ensuring a continuity between the development and business teams.

**Camille Delgrange** was the head of the bioassay team, investigating how to best adapt our chosen technology to the plasma matrix and optimizing the overall bioassay protocol. She also made sure deadlines were kept and medal submissions were done on time. During the semester, she also investigated an electrochemical aptasensor technique.

**Ahmed Emam** was our creative director and thus in charge of the promotion of the team. He notably worked on the instagram take-over and the pitch.

**Marco Fumagalli** was our very own Swiss knife. He was in charge of designing and printing the reader system and took care of the microscope illumination set up.

**Zeyd Ghomri** was part of the business team, ensuring the financial viability of the project through cost projections, revenue streams and market size. He also worked on the Business model. Furthermore, he was our community manager, regularly posting updates throughout the project.

**Mateo Hamel (Team Captain)** was part of the business team, contacting sponsors and investigating the stakeholder desirability. He also worked on the financial viability of our project and ensured the communication with our supervisor and continuity between the development and business teams.

**Gaiëtan Renault** was part of the software team, working on the autofocusing algorithm and linking the soft - and the hardware with a GUI.

**Imane Wifak** was part of the bioassay team. She participated in the optimization of the overall bioassay protocol. She also investigated the optimal pairs of antibodies based on bio-layer interferometry.

**Karim Zahra (Team Captain)** was part of the business team, giving us valuable insights on the needs of the targeted market. He also worked on the financial viability of our project and ensured the communication with our supervisor and continuity between the development and business teams.

## 6.2 Support

We are thankful for the support of **Prof. Philippe Renaud**, **Prof. Hatice Altug** and **Dr. Arnaud Bertsch** throughout this project. They provided us with valuable advice to overcome encountered problems during the project. **Abtin Saateh** greatly helped us in the development process, always having great advices and an open ear for when we needed him. Additionally, we thank Dr. Florence Pojer and Kelvin Lau from the Protein Production and Structure Core Facility that helped us use their biolayer interferometry (BLI) machine to test our antibody pairs. We also thank Micaela Siria Cristofori for her help in analyzing the BLI results, Saeid Ansaryan for his help on the bioassay and the chips spotting. We also received support from people outside of EPFL such as Dr. Sylvain Meylan, head of the Sepsis research program of the Lausanne University Hospital, Dr. Yasmine Gamal, Dr. Angela Bikker, Eric Meurville, Dr. Hossam Ammar, Dr. Basma Shehata, Roberto Constantini, Mohamed Kedr, Dr. Judy Fonville, Senior Scientist Clinical Research and Statistics at Siemens Healthineers and Ir. Toon Stilma, lead at Roland Berger Tenzing. We finally thank Théo Mayer, William Verstraeten and Janet van der Graaf Mas from last year's team that introduced us to the competition and helped us when we had questions about it.

**Sponsors**

We are most grateful for the support of mutiple sponsors during this project.

**Thorlabs** provided us with the optical components of our system.

**AMF Medical** provided us with a pump for our microfluidics system and supported us in resolving pump issues.

**COMSOL Multiphysics** provided us with licenses allowing us to perform microfluidics simulations to improve our system.

**Forum EPFL** offered us finanical support.

**EPFL** provided us with the necessary framework to develop our sensor, granting us access to laboratories, meeting rooms and to needed machines and materials.

# 7 Final Remarks

Our main motive for coming together as a team and founding Lau'Sens was to ultimately change people's lives for the better and help those who need it most. With acute inflammation and especially Sepsis, we have found the need for a biosensor like ours to not be inside our nation but rather across the Mediterranean. But since diseases are not constrained by borders, we believe our technology should be outsourced internationally as well. We hope these pages managed to convince you not only of the feasibility and innovation of our biosensor but also of our eagerness to contribute in managing this urgent global health concern. We are currently working on obtaining further data with our final prototype, determining our LoD, and acquiring our final calibration curve for the competition. We are also aiming to integrate everything in an even easier way so that the user will only need to put the sample vial into the reader and wait for the result. As a final word, we want to use this opportunity to thank all the people that helped and supported us during these last few months. We also want to acknowledge the SensUs Organization Team for making this competition possible.

We are looking forward to the SensUs Innovation Days in Eindhoven to present our biosensor, meet the other teams and create lifelong memories!

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## Interviews

[1] Interview with Dr. Yasmine Gamal - Pediatric Nutrition Consultant & Professor at the Faculty of Medicine - Ain Shams University, Cairo, Egypt. 27 July 2022.

[2] Interview with Dr. Hossam Ammar - Consultant of Neonatology and Pediatric Hospitals of the Faculty of Medicine - Ain Shams University, Cairo, Egypt. 24 July 2022.

[3] Interview with Dr. Basma Shehata - Lecturer of Pediatrics and Neonatology, Faculty of Medicine - Ain Shams University, Cairo, Egypt. 4 August 2022.

[4] Interview with Dr. Angela Bikker - Laboratory Specialist Clinical Chemistry, Unilabs and St. Antonius Ziekenhuis. 29 July 2022.

[5] Interview with Roberto Constantini - Medical Device Consultant at Elseemed Sarl - Project Management Professional. 3 August 2022

[6] Interview with Mohamed Khedr - CEO of Techno Wave. 2 August 2022.
[7] Interview with Eric Meurville - Technology acceleration manager at Technology Transfer Office
[8] Interview with Mohamed Khedr - CEO of Techno Wave. 2 August 2022.
[9] Interview with Dr. Sylvain Meylan - Chief of sepsis project in CHUV.

# 8 Appendix

## 8.1 Biosensor system and assay

### 8.1.1 Reader and Graphical User Interface

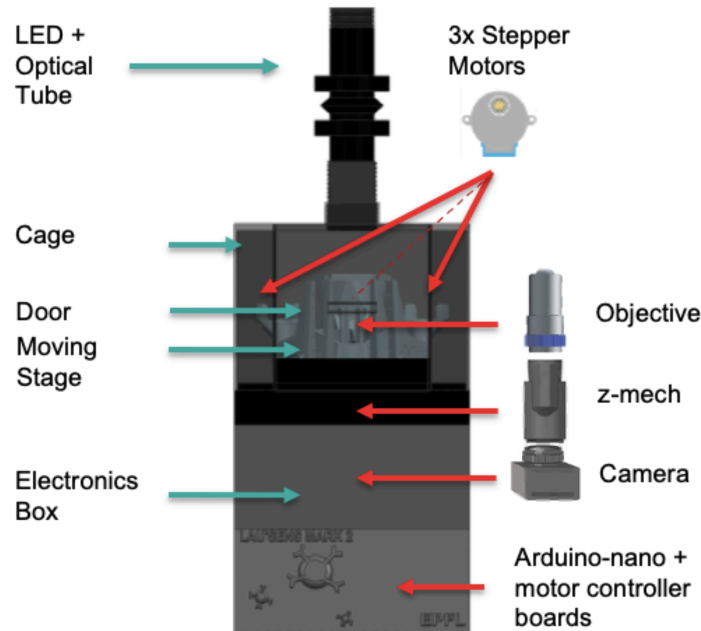



**Figure 7: LauSens Reader:** Starting from the top, we have LED illumination, followed by the optical tube. Stepper motors are fixed on the structures for x-y-z movement. The objective and camera are mounted on the Optical Tube and fixed to the z-moving mechanism through it. The core structure has been modified to contain the camera and motor controller boards.

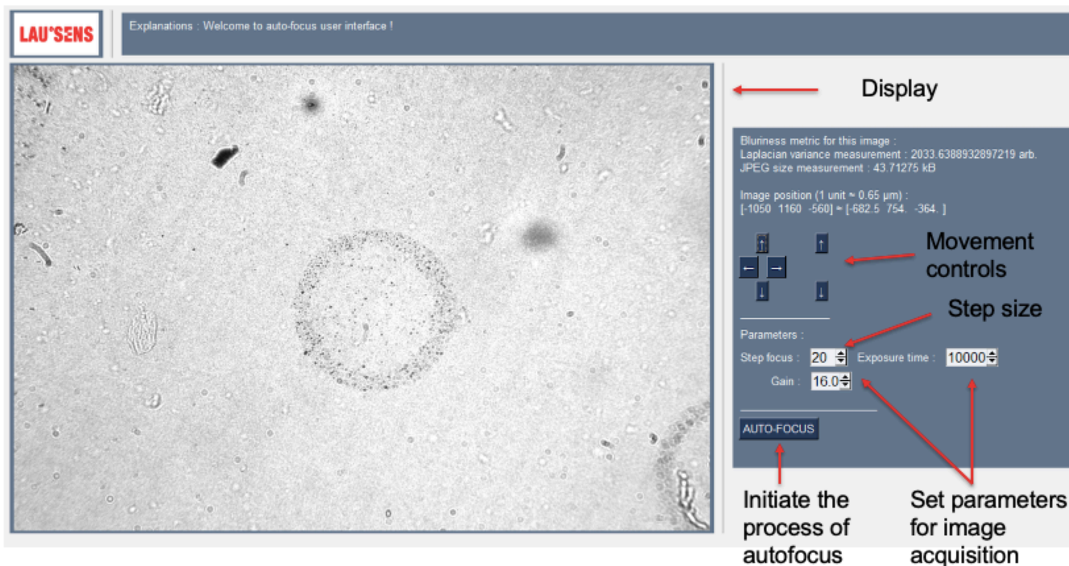



**Figure 8: GUI to control the LauSense reader:** The graphical interface allows for fine movements across the surface of the chip and in the z direction using just the mouse. Once the region of interest is at the center of the field of view, the autofocus procedure can be started by clicking on the AUTO-FOCUS button.

## 8.2 Technical feasibility

### 8.2.1 Molecular recognition and assay reagents

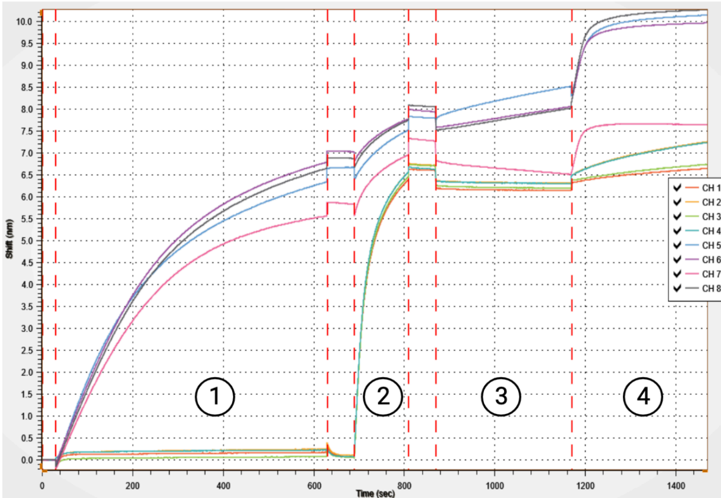



**Figure 9:** Representation of the wave shift signal across time of the different sandwich pairs (BLI): 1: First (capture) antibody binding. 2: Blocking solution signal to avoid non-specific binding. 3: Antigen binding (IL-6). 4: Second (detection) antibody binding. CH1 to CH8 represent the different sandwich pairs.

## 8.3 Business Model

**PROBLEM**

- A high proportion of neonates wrongly suspected of sepsis (not reliable early diagnosis methods for Neonatal Sepsis (NS))
- When sepsis is suspected, reaction time is critical
- Limited resources in LMICs (overcrowded NICUs, second-hand medical equipment, shortage of healthcare professionals)

**EXISTING ALTERNATIVES**

- Misuse/Overuse of Antibiotic Treatments (ABT)
- Diagnosis based on clinical symptoms
- Laboratory tests (CRP, PCT, manual blood cultures) expensive, not accurate or with long time to result.

**SOLUTION**

- Syndromic test for early sepsis diagnose
- Multiplex solution, giving no false negative results (reduce ABT overuse for neonates)
- Microfluidic cartridge allowing short time-to-result
- User-friendly interface for easy manipulation
- Cost-effective in vitro device

**KEY METRICS**

- 62,5% specificity and 100% sensitivity
- 2/3 of neonates with suspected sepsis prevented from undergoing unnecessary ABT
- 10% of the market size (LMIC's NICU) reached in 15 years

**UNIQUE VALUE PROPOSITION**

- 7'648CHF saved yearly per NICU (156'000 EGP)
- 46 neonate ABTs avoided yearly per NICU (a total of 190'000 in Egypt)
- Product as a service (PaaS) including automatized cartridge supply, maintenance and on-field technical support

**HIGH-LEVEL CONCEPT**

- Antigenic Covid-19 test for NS

**UNFAIR ADVANTAGE**

- Spinoff EPFL (already existing patent, funding, mentoring)
- Favourable Network: NICU Specialists (and other health-care professionals) in Cairo

**CHANNELS**

- Governmental insurance (i.e. Tamin Sehy)
- NGO support (such as Red Cross and Egyptian Neonatal Network)
- International medical exhibitions and conferences, such as InterLab Africa and Africa healthOutsourced sales force
- Word of mouth in medical world

**CUSTOMER SEGMENTS**

- Neonatal Intensive Care Units (NICUs) of public hospitals all over Egypt
- NICU of private hospitals all over Egypt
- NICU all over LMIC's

**EARLY ADOPTERS**

- NICU of Ain Shams public hospital (Cairo, Egypt)
- NICUs of others public hospitals (Cairo, Egypt)

**COST STRUCTURE**

- Fixed costs: regulation, IP rights, R&D, payroll, marketing, infrastructures, consultancy
- Variable costs: cost of goods sold (including cost of insurance and freights, manufacturer margin, ...), agent commissions, IP right EPFL licensing

**REVENUE STREAMS**

- PaaS with the following costs per NICU (with the possibility of monthly payments):
  - 7'900 CHF/year for a 1-year contract
  - 7'655 CHF/year for a 3-year contract
  - 7'409 CHF/year for a 5-year contract

**Figure 10:** Business model

## 8.4 Business feasibility

### 8.4.1 Device monitoring

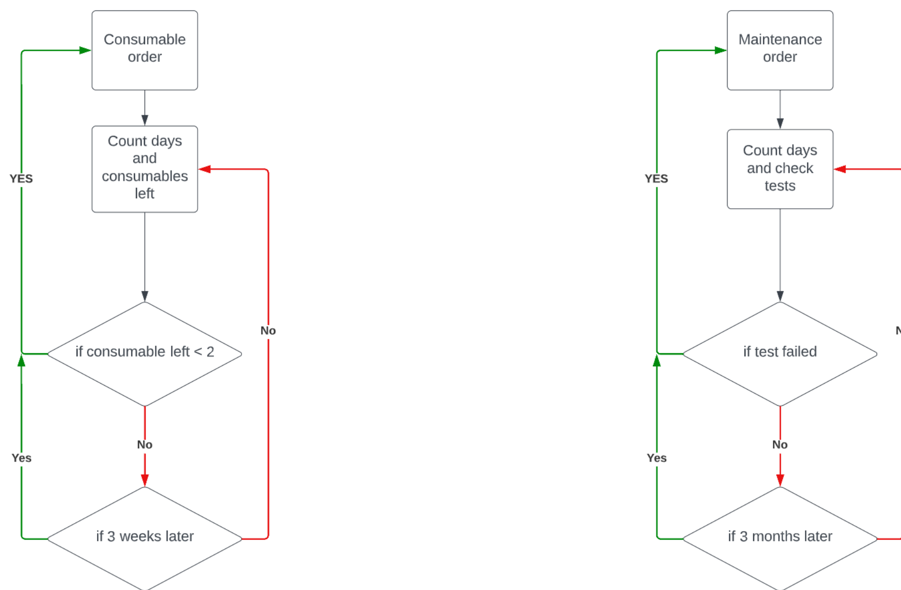



**Figure 11:** Device monitoring for consumable and maintenance automatization

### 8.4.2 Risk assessment

| Risk assessment for proposed biosensor | | | | | | | |
|---|---|---|---|---|---|---|---|
| Risk ID | Category | Hazard | Hazardous situation | Severity | Occurrence | Risk | Mitigation |
| 1 | Reader | Electrical Energy | User suffers electric shock while plugging/unplugging sensor | 2 | 1 | 2 | Insulate all electronic parts |
| 2 | Reader | Mechanical failure | Part of reader breaks | 1 | 2 | 2 | Offer customer support, have independent, replacable parts and cooperate with geographically close company able to undertake repairs. Include checkpoints on the assay and safe logs for each test to conduct error management. |
| 3 | Microfluidics | Mechanical failure | Pump breaks, leaving the system unusable | 1 | 1 | 1 | Offer customer support, have independent, replacable parts and cooperate with geographically close company able to undertake repairs. Include checkpoints on the assay and safe logs for each test to conduct error management. |
| 4 | Microfluidics | Mechanical failure | Liquid remains in microfluidic channels | 1 | 2 | 2 | Have independently replacable microfluidics parts, cooperate with geographically close company able to undertake repairs. Include checkpoints on the assay and safe logs for each test to conduct error management. |
| 5 | Microfluidics | Leakage | The sample exits the cartridge, with the possibility of contamination to user and device. If the sample stays in the device, it could impact the next test and give a false positive result. | 3 | 1 | 3 | Control strategies in manufacturing so that any defective cartridge never reaches the market. Use of disposable latex gloves and mask when manipulating the cartridge. Cleaning of the reader and microfluidics cartridge if any leakage is suspected. |
| 6 | Software | Data Analysis | Implemented algorithm encounters bug and gives faulty result | 3 | 1 | 3 | Implement multiple safety levels and error messages in code. Call an invalid result when a software error message appears. |
| 7 | Sample Preparation | Biological agent | Analyzed blood sample is contaminated and transfers contamination to user during sample preparation | 3 | 1 | 3 | Including safety measures & warnings in the user manual such as wearing gloves when handling samples |
| 8 | Bioassay | Functionality | False callings due to non functional array leading to neonate being sent home while having sepsis | 3 | 2 | 6 | Include internal control (e.g. Heparin) to distinguish between invalid and true negative tests |

**Figure 12:** Product risk analysis and management.

| | | Severity | | |
|---|---|---|---|---|
| | | 1 (low) | 2 (middle) | 3 (high) |
| Occurrence | 1 (low) | 1 | 2 | 3 |
| | 2 (middle) | 2 | 4 | 6 |
| | 3 (high) | 3 | 6 | 9 |

**Figure 13:** Risk matrix, explaining different levels of severity and occurrence.

### 8.4.3 Long term suppliers

| Type | Partners | Products |
|---|---|---|
| **Cartridge** | ABCAM | Antibodies |
| | CYTODIAGNOSTICS | Au-NPs |
| | UCSB NANOFABRICATION | Chips |
| | SCIENION CELLENION | Cellen1 Micro spotting |
| | THERMOFISCHER | Lab chemicals (buffer, heparin, NaOH...) |
| | LUCIDANT | Polymer MCP-2 |
| | CMI EPFL | Channel PDMS |
| **Biosensor** | AMF | Micro pump |
| | THORLABS | Optics |

**Figure 14:** Key suppliers

### 8.4.4 Sustainability plan

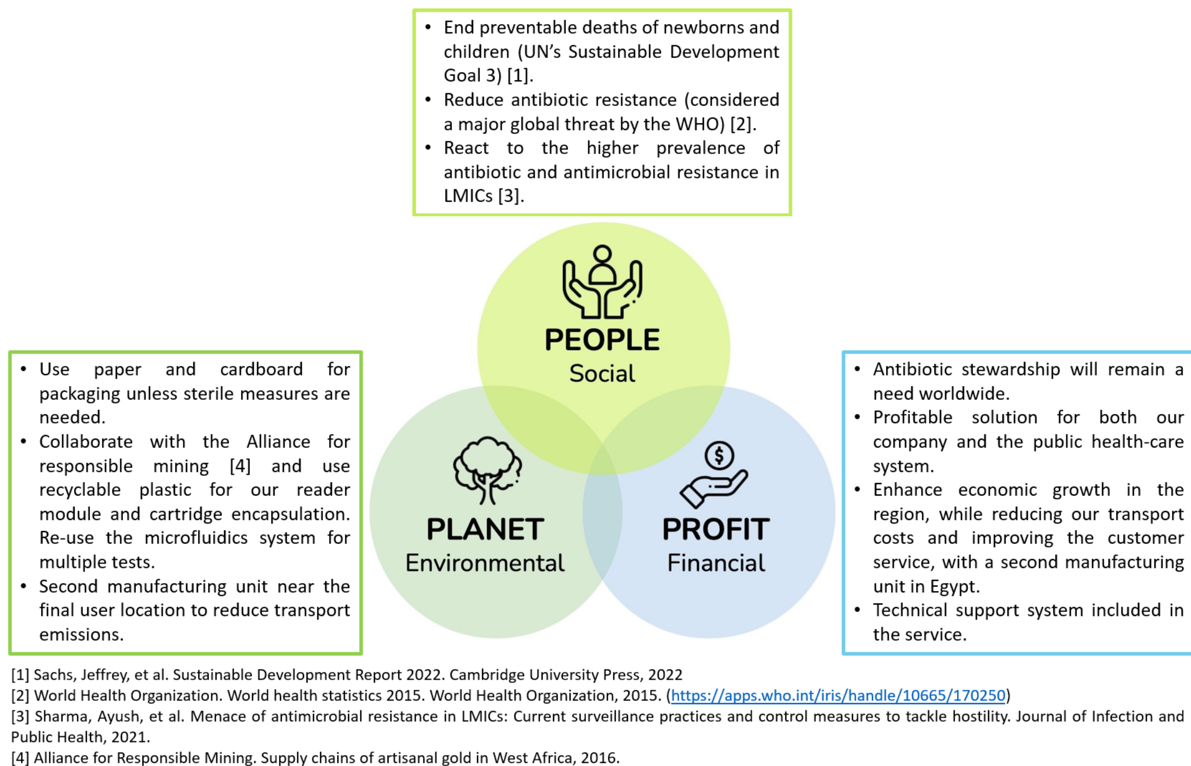



**Figure 15:** The three pillars of sustainability for LauSens.

## 8.5 Financial viability

### 8.5.1 Biosensor and consumables cost

| Components | Number | Price (CHF/unit) | Price (CHF) |
|---|---|---|---|
| **Microfluidics** | | | **2005,6** |
| AMF pump | 1 | 2000 | 2000 |
| channel PDMS | 1 | 5 | 5 |
| MF holders 3D printed | 10 | 0,06 | 0,6 |
| **Optics** | | | **1884,83** |
| Mounted LED | 1 | 217,78 | 217,78 |
| camera | 1 | 800 | 800 |
| Objective RMS40X | 1 | 653,33 | 653,33 |
| lense | 2 | 23,42 | 46,84 |
| diaphragm | 2 | 83,44 | 166,88 |
| **Structures & Translation Stage** | | | **129,3** |
| Motor stepper | 3 | 18,7 | 56,1 |
| Mounting kit | 1 | 37,2 | 37,2 |
| Printed parts | 600 | 0,06 | 36 |
| **Electronics** | | | **252,8** |
| Arduino nano | 1 | 35,1 | 35,1 |
| Raspberry Pi | 1 | 74 | 74 |
| Touchscreen for RP | 1 | 88 | 88 |
| Jumper wires kit | 1 | 17,9 | 17,9 |
| Traco power TXM 025-105 | 1 | 25,8 | 25,8 |
| Schurter DD11 | 1 | 12 | 12 |
| **Packaging** | | | **100** |
| | | **PROTOTYPE PRICE:** | **CHF4 372,53** |
| | | **OPTIMIZED PRICE:** | **CHF2 929,60** |

**Figure 16:** Biosensor cost

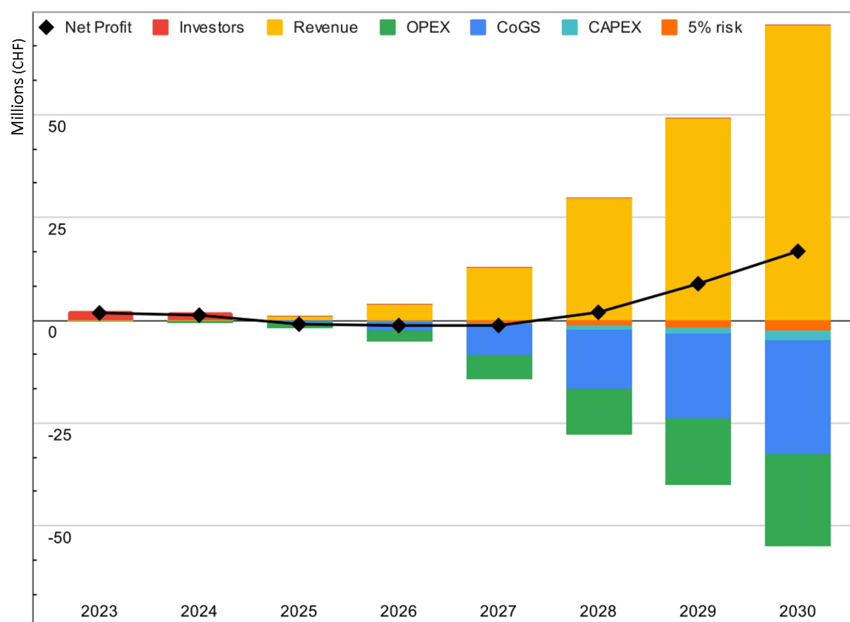
| Components | Volume | Units | Price (CHF/units) | Price (CHF) |
|---|---|---|---|---|
| **Au-NPs Functionalization** | **100** | **uL** | **0,685** | **68,5** |
| Detector AB anti AB IL-6 | 12,5 | ug | 1,52 | 19 |
| Au-Nps | 100 | uL | 0,445 | 44,5 |
| Lab chemicals | | | | 5 |
| **Chip** | **200** | **units** | **4,625** | **925** |
| Capture AB anti AB IL-6 | 50 | ug | 1,52 | 76 |
| MCP-2 polymere | 140 | uL | 0,35 | 49 |
| Wafer | 1 | unit | 800 | 800 |
| **Packaging** | | | | **0,56** |
| Chips package | 1 | unit | 0,4 | 0,4 |
| Au-NPs functionnalized package | 1 | unit | 0,16 | 0,16 |
| **Total** | | | | |
| PBS | 7 | uL | 0,0000696 | 0,0004872 |
| Heparin | 25000 | ug | 0,000193 | 4,825 |
| NaOH | 2,5 | uL | 0,00002275 | 0,000056875 |
| Tween 20 | 0,5 | uL | 0,000075 | 0,0000375 |
| Au-NPs functionnalized | 2,5 | uL | 0,685 | 1,7125 |
| Super Block Blocking Buffer | 20 | uL | 0,0006825 | 0,01365 |
| Chip | 1 | unit | 4,625 | 4,625 |
| | | | **CONSUMABLE'S PRICE:** | **CHF11,18** |
| | | | **OPTIMIZED PRICE:** | **CHF7,49** |

**Figure 17:** Consumable cost

### 8.5.2 Profit and Loss

| Lau'Sens<br>Profit and Loss (P&L) Statement | | Proof of Concept | Cairo | | Egypt | | Other LMICs | | Gray value are complementary information | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 | 2029 | 2030 | 2031 | 2032 |
| **Expenses** | | **341 129** | **738 072** | **1 734 217** | **4 851 648** | **13 486 614** | **26 440 838** | **38 310 189** | **52 337 517** | **68 950 429** | **81 534 394** |
| **Expenses (5% for uncertainty)** | | **358 185** | **774 975** | **1 820 928** | **5 094 231** | **14 160 945** | **27 762 880** | **40 225 698** | **54 954 393** | **72 397 951** | **85 611 114** |
| **CAPEX (Capital Expenses)** | | **33 700** | **1 700** | **27 156** | **115 821** | **394 250** | **892 433** | **1 474 465** | **2 153 385** | **2 904 330** | **3 411 164** |
| | IP rights licensing EPFL (3%) | 0 | 0 | 25 456 | 114 121 | 386 450 | 890 613 | 1 472 625 | 2 151 525 | 2 902 450 | 3 409 244 |
| | IP rights - patent | 2 000 | 0 | 0 | 0 | 6 100 | 120 | 140 | 160 | 180 | 220 |
| | CE (EU IVDR 2017/746) | 30 000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Total FSC | 1 700 | 1 700 | 1 700 | 1 700 | 1 700 | 1 700 | 1 700 | 1 700 | 1 700 | 1 700 |
| **CoGS (Cost of Goods Sold)** | | **56 114** | **140 286** | **634 568** | **2 703 745** | **8 549 789** | **17 099 832** | **24 173 592** | **32 697 839** | **43 276 252** | **51 411 963** |
| | **Government support included (15% discount)** | **47 697** | **119 243** | **539 383** | **2 298 183** | **7 267 320** | **14 534 857** | **20 547 553** | **27 793 163** | **36 784 814** | **43 700 168** |
| | Biosensor components | 2 930 | 2 930 | 2 930 | 2 930 | 2 930 | 2 930 | 2 930 | 2 930 | 2 930 | 2 930 |
| | # of biosensors (5% loss) | 10 | 25 | 113 | 470 | 1 438 | 2 649 | 3 369 | 4 438 | 5 551 | 6 663 |
| | Consumable components | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| | # of consumable (average of 168 per BS per year) | 1 680 | 4 200 | 18 998 | 79 029 | 241 536 | 445 075 | 566 008 | 745 558 | 932 503 | 1 119 449 |
| | cumulative # of consumable | 1 680 | 4 200 | 18 998 | 85 413 | 289 584 | 667 675 | 1 091 008 | 1 522 390 | 2 141 263 | 2 516 705 |
| | Total components costs | 41 876 | 104 691 | 473 558 | 2 017 720 | 6 380 439 | 12 761 069 | 18 039 994 | 24 401 373 | 32 295 710 | 38 367 136 |
| | Cost insurance and freight - CIF (20%) | 8 375 | 20 938 | 94 712 | 403 544 | 1 276 088 | 2 552 214 | 3 607 999 | 4 880 275 | 6 459 142 | 7 673 427 |
| | VAT in Egypt (taxes) and custom duties (14%) | 5 863 | 14 657 | 66 298 | 282 481 | 893 261 | 1 786 550 | 2 525 599 | 3 416 192 | 4 521 399 | 5 371 399 |
| **OPEX (Operating Expenses)** | | **259 731** | **617 128** | **1 167 679** | **2 437 645** | **5 825 044** | **11 013 548** | **16 288 171** | **22 390 969** | **29 261 285** | **34 423 062** |
| | Agent commissions (25% of revenues) - Technowave | 0 | 0 | 212 130 | 951 004 | 3 220 413 | 7 421 774 | 12 271 872 | 17 929 373 | 24 187 086 | 28 410 363 |
| | Contract manufacturer gross margin (7%) | 2 931 | 7 328 | 33 149 | 141 240 | 446 631 | 893 275 | 1 262 800 | 1 708 096 | 2 260 700 | 2 685 700 |
| | R&D budget | 200 000 | 250 000 | 50 000 | 50 000 | 50 000 | 50 000 | 100 000 | 100 000 | 150 000 | 150 000 |
| | Payroll | 0 | 300 000 | 800 000 | 1 200 000 | 2 000 000 | 2 500 000 | 2 500 000 | 2 500 000 | 2 500 000 | 3 000 000 |
| | Marketing (website, exhibitions, ...) | 5 000 | 10 000 | 10 000 | 30 000 | 30 000 | 30 000 | 50 000 | 50 000 | 60 000 | 60 000 |
| | Number of employees | 12 | 12 | 16 | 16 | 20 | 25 | 25 | 25 | 25 | 30 |
| | Infrastructures (3900CHF / person) | 46 800 | 46 800 | 62 400 | 62 400 | 78 000 | 97 500 | 97 500 | 97 500 | 97 500 | 117 000 |
| | Consultancy | 5 000 | 0 | 0 | 0 | 0 | 15 000 | 0 | 0 | 0 | 0 |
| | Audit | 0 | 3 000 | 0 | 3 000 | 0 | 6 000 | 6 000 | 6000 | 6 000 | 0 |
| **Profits** | | **2 130 000** | **2 000 000** | **848 520** | **3 804 018** | **12 881 653** | **29 687 094** | **49 087 486** | **71 717 490** | **96 748 342** | **113 641 451** |
| **Investors** | | **2 130 000** | **2000000** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| | EPFL tech launchpad ignition | 30 000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | EPFL Innogrants | 100 000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Venture Capitals | 1 500 000 | 2000000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Angel investors | 500 000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Revenues** | | **0** | **0** | **848520** | **3804018** | **12881653** | **29687094** | **49087486** | **71717490** | **96748342** | **113641451** |
| | # of 1y-subscription | 0 | 0 | 70 | 200 | 300 | 500 | 600 | 1000 | 2000 | 2500 |
| | yearly fee | 7902 | 7902 | 7902 | 7902 | 7902 | 7902 | 7902 | 7902 | 7902 | 7902 |
| | # of 3y-subscription | 0 | | 30 | 200 | 700 | 1000 | 1800 | 2000 | 1280 | 2500 |
| | cumulative # of subscription | 0 | 0 | 30 | 230 | 930 | 1900 | 3500 | 4800 | 5080 | 5780 |
| | 3-years fee | 7804 | 7804 | 7804 | 7804 | 7804 | 7804 | 7804 | 7804 | 7804 | 7804 |
| | # of 5y-subscription | 0 | | 8 | 48 | 369 | 1000 | 800 | 1226 | 2000 | 1346 |
| | cumulative # of subscription | 0 | 0 | 8 | 56 | 425 | 1425 | 2225 | 3443 | 5395 | 6372 |
| | 5-years fee | 7655 | 7655 | 7655 | 7655 | 7655 | 7655 | 7655 | 7655 | 7655 | 7655 |
| **NET PROFIT (CHF)** | | **1 771 815** | **1 225 025** | **(972 408)** | **(1 290 213)** | **(1 279 292)** | **1 924 214** | **8 861 788** | **16 763 098** | **24 350 392** | **28 030 337** |

**Figure 18:** Profit and Loss statement




Estimated Profits and Loss from 2023 to 2030

**Figure 19:** Expected Net Profit over the years

### 8.5.3 Sales price

| Lau'Sens | | | | |
|---|---|---|---|---|
| Sales Price | | | Suscription | |
| | | 1y contract | 3y contract | 5y contract |
| **Expenses** | | **6 994** | **6 970** | **6 933** |
| **COGS** | | **5 905** | **5 905** | **5 905** |
| | **Government support included (15% discount)** | **5 019** | **5 019** | **5 019** |
| | Biosensor components | 2 930 | 2 930 | 2 930 |
| | Consumable components | 7 | 7 | 7 |
| | # of consumables (average of 168 per BS per year) | 168 | 168 | 168 |
| | Total components costs | 4 188 | 4 188 | 4 188 |
| | Cost insurance and freight - CIF (20%) | 838 | 838 | 838 |
| | VET in Egypt (taxes) and custom duties (14%) | 586 | 586 | 586 |
| | Contract manufacturer margin (7%) | 293 | 293 | 293 |
| **OPEX** | **Agent commissions (25% of revenues) - Technowave** | **1 976** | **1 951** | **1 914** |
| **Yearly Fee** | | **7902,4** | **7803,62** | **7655,45** |
| | COGS | 4939 | 4939 | 4939 |
| | Margin (agents gross margin included) | 60% | 58% | 55% |
| **MARGIN** | | **11%** | **11%** | **9%** |
| **PROFIT** | | **908** | **834** | **723** |

**Figure 20:** Sales price analysis

| Actual Procedure | |
|---|---|
| **Medical Care per neonates** | **Daily Costs** |
| Care in the NICU | 75 |
| Laboratory Tests (blood cultures 13CHF,...) | 2 |
| Antibiotics Treatment | 8 |
| **Daily cost** | **CHF84,50** |
| Average duration of treatment (days) | 7 |
| **Total cost per neonates** | **CHF592** |

| Target Procedure | |
|---|---|
| Monthly suscription fees | 659 |
| Number of baby tested monthly | 8 |
| Proportion of negative per suspected neonates | 72% |
| **Total cost per neonates** | **CHF512** |
| **Monetary NICU Benefit per neonates** | **CHF79** |
| **Yearly Monetary NICU Benefit** | **CHF7 648** |
| **Yearly Government Benefit** | **CHF33 652 123** |
| **Number of ABT avoided per year** | **189 539** |

**Figure 21:** Unique value proposition

### 8.5.4 Market size

| Market Share | Cairo | | Egypt | | Africa | |
|---|---|---|---|---|---|---|
| | # of NICU | Yearly Revenue | # of NICU | Yearly Revenue | # of NICU | Yearly Revenue |
| 2,00% | 22 | 193 775 | 88 | 792 000 | 1 501 | 13 508 639 |
| 10,00% | 108 | 968 876 | 440 | 3 960 000 | 7 505 | 67 543 197 |
| 50,00% | 538 | 4 844 382 | 2 200 | 19 800 000 | 37 524 | 337 715 985 |
| 80,00% | 861 | 7 751 011 | 3 520 | 31 680 000 | 60 038 | 540 345 576 |

**Figure 22:** Addressable Market size