BiosensUM

Team Results Document

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1.Summary

We have developed an all-in-one biosensor based on a competitive fluorescent assay for the quantitative measurement of the free fraction of valproate (fVPA) in blood in the therapeutic range of 1.0 to 15.0 µg/mL (~10 % of total VPA). The procedure is completed in less than 5 minutes, starting from a crude blood sample of only 0.7 µL injected into a disposable cartridge inserted in a user-friendly, 8.0x7.0x4.0 cm portable device. fVPA is separated from the bound fraction thanks to a robust microdialysis system and is then autonomously combined with its fluorescent derivative and guided into a reaction chamber where the assay takes place through antibody binding. Laser-triggered fluorescence signal is electronically processed to offer a concentration value computed from adequate image processing and prior calibrations, providing test results conveniently displayed onto a digital screen. Complementary calculations have confirmed that the technology is easily adaptable to various targets through slight cartridge adaptations, a versatility highly sought-after by health professionals interviewed through our market study. Thorough modelizations estimate the production cost of our cartridge and sensor to be of 7.71-10.28 and 260-460 CAD, respectively, depending on production scale. Carefully built financial plan predicts profitability starting from year 4 of our startup.

2. Biosensor system and assay

We have developed an all-in-one biosensor based on a competitive fluorescent assay for the quantitative measurement of the free – unbound – fraction of valproate (fVPA) in blood in the therapeutic range of 1.0 to 15.0 μg/mL (~10 % of total VPA). In less than 5 minutes, with a crude blood sample of only 0.7 µL and without any pre-treatment, the free fraction of the drug is separated from the bound fraction and larger blood proteins by diffusion through a microporous membrane, thanks to a robust microdialysis system, and is then collected in a phosphate-buffered saline (PBS) solution. fVPA is then combined to a known quantity of its fluorescent derivative, fluorescein isothiocyanate-labelled VPA (VPA-FITC), also dissolved in PBS, and the resulting mixture is transported to the reaction chamber where the fluorescence assay takes place. Competitive binding to anti-VPA antibodies previously immobilized onto a glass surface can then take place. After a reaction time of 3 minutes, the mixture is gradually exchanged for a washing solution provided by a reservoir, in order to remove any residual analyte or fluorescent derivative that has not bound to the antibodies. When the washing step is completed, the optically transparent reaction surface is irradiated from below using a 495 nm wavelength (λ_{ex}) to excite the FITC moiety. Afterwards, a camera also positioned under the surface captures the resulting fluorescence signal at 525 nm, which corresponds to the emission wavelength of the fluorescent moiety (λ_{em}). The signal is then electronically processed to offer a concentration value computed from adequate image processing and prior calibrations. Our biosensor not only allows for the monitoring of fVPA in blood, but also for that of other commonly used antiepileptic drugs, such as carbamazepine (CBZ) and phenytoin (PHT). These molecules are associated with well-known antibodies and can be functionalized with a FITC group relatively easily, making them suitable for fluorescence assays involving virtually the same excitation and emission wavelengths. We thus propose a versatile technology for the monitoring of fVPA and other common anticonvulsants, thanks to interchangeable all-in-one cartridges, which contain antibodies for either VPA, CBZ, or PHT. In this document, we will mainly refer to VPA to describe the operating details of our technology, but the principles remain closely similar for CBZ and PHT (details given in Appendix A).

2.1 Molecular recognition and assay reagents

In order to specifically detect the free fraction of the target molecule, anti-VPA IgG1 monoclonal antibodies are immobilized on a small surface, whose dimensions fit that of the reaction chamber, following a general procedure for glass, silica, and quartz surfaces suggested by Thermoscientific.[1] It first involves the aminosilylation of the surface, followed by the maleimide activation of the resulting amino groups, the addition of sulfhydryl groups to the antibodies for coupling, and finally, the cross-linking of the sulfhydryl-functionalized antibodies to the activated surface. To allow the competitive bioassay to take place, the VPA-FITC is synthesized by dissolving an equivalent mass of 2-propyl-6-aminohexanoic acid and fluorescein isothiocyanate isomer 1 in methanol containing 1 mL/L of triethylamine. After stirring for 2 h, in the dark, at ambient temperature, the solution is acidified with 1 M HCl and it is then filtered. The resulting precipitate is then dissolved in methanol and can be easily purified using only thin-layer chromatography. Following this method, the major band ($R_f = 0.3$) is then scraped from the plate, extracted with methanol and stored in a freezer at -20 °C. The concentration of the fluorescein tracer can then be estimated spectrophotometrically.[2,3]

2.2. Physical transduction

Our physical transduction lies on the conversion of a light signal into an electrical signal. This analytical technique enables our label-based biosensor to provide an efficient real-time response. In this context, we can achieve a highly specific detection and yet minimize the number of components, thus proposing a simple, easy to implement, and powerful signal transduction. As a matter of fact, our system is composed of only 6 components: a laser diode, a dichroic filter, a lens, a laser filer, a photodiode and a microcontroller board. The laser beam irradiates an area of the reaction chamber by passing through a dichroic filter and hitting a converging lens which focusses the incident light. Since polydimethylsiloxane (PDMS) is optically transparent, the light passes through the cartridge walls and interacts with the molecules bound to the antibody functionalized surface. The luminous signal strength resulting from the emission of the FITC moiety is inversely proportional to the amount of fVPA in the reaction chamber. Before being converted into an electrical signal, the emitted light is directed towards the photodiode using the dichroic filter. To ensure that no optical pollution from the laser reaches the photodiode, a second filter guarantees that only wavelengths over 500 nm is detected. The photodiode then sends an analog signal to the microcontroller board.

2.3. Cartridge technology

Our biosensor operates with a disposable multi-chamber glass and PDMS cartridge incorporating a dialysis chamber and multiple flow-controlling valves, shown in Figure 1. The blood sample is introduced into the cartridge through a micro-channel by capillary action similar to how blood glucose test strips get filled. This creates a diffusion gate that preferentially lets smaller molecules like fVPA migrate faster through the microporous membrane than larger molecules such as proteins and protein-bound VPA, thanks to their size and greater diffusion coefficient. This key and distinctive feature efficiently separates the free VPA from the bound fraction and allows to precisely measure the fVPA blood concentration further down the chip's microfluidic circuit. Well-studied diffusion equations enable the estimation of the time required for the quantitative migration of the fVPA to take place. Furthermore, since a similar approach has successfully been used in a recent article,[4] we were able to make use of empirical results to adjust our theoretical estimates. While the diffusion takes place, the fluid is static, held back by surface tension forces. After the estimated time for migration is reached, the microcontroller induces an electrical current through a cathode/anode pair, both in contact with the fVPA-filled PBS buffer solution, thus initiating an electrolysis process. This generates two gas bubbles (H_2 and O_2), one at each electrode, effectively pushing the liquid forward by breaking the capillary pressure barrier. The fVPA-filled liquid then reaches the Y junction (Figure 1 between A and B), triggering a liquid-controlled microvalve. The simultaneous presence of two liquids at the junction, the fVPA-filled solution and the VPA-FITC solution, allows for the mixing and the further progression of the two fluids into the reaction chamber, where the competitive assay takes place. Once the competitive binding has taken place, the washer solution, which is pushed by an electrolysis bubble, floods the reaction chamber and effectively forces out the unbound molecules into the waste chamber, allowing the physical transduction to take place. For the details of the cartridge manufacturing process, see Appendix B.

2.4. Reader instrument and user interaction

The reader instrument has the shape of a trapezoidal prism on top of a rectangular base, as shown Figure 1 (See dimensions in Appendix C). The disposable cartridge is inserted through a slot located on top of the device. One of the inclined sides features the main elements of the user interface: a button which allows the user to turn the device on and off and a touchscreen which controls the initiation of the analysis and that displays assay instructions and test results. As soon as the device is turned on, instructions on how to correctly collect the blood sample and insert the cartridge are shown. When the analysis is completed, the results appear on the screen and the cartridge is pushed up by a small motor for the user to remove and discard it. The analog signal received by the microcontroller from the photodiode is compared to the internally saved calibration curves which will be established precisely in our labs. This key step ensures the analytical performance of our biosensor and a lot of effort will be put into establishing complete calibration curves.

The microcontroller used can be connected to the Internet through a wireless transceiver. Down the road, this would give every device the ability to communicate with the data system of the care center through a secure and encrypted connection. This feature can be implemented on already sold devices through simple updates of the software. Once the device is set up, the care provider only needs to access the patient's file through its number and the results will not only be shown on the screen but also sent to the digital patient file. This was a highly sought-after feature mentioned during our interviews as it facilitates the communication between different health professionals and enables them to have a better understating of the patient's situation. The whole procedure was designed to last less than 5 minutes and to optimize ease of use.

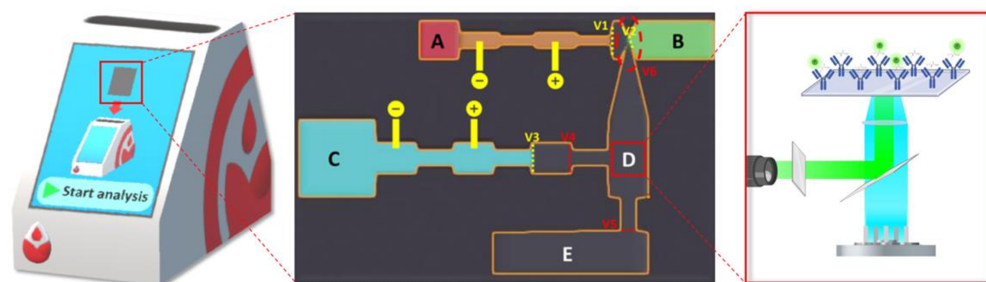


Figure 1: (left) Exterior of our biosensor with the power button and touch screen in the front and the cartridge compartment on top. **(right)** Schematic representation of our cartridge. **A:** dialysis chamber pre-filled with PBS, the blood sample is introduced in the upper chamber, over a porous membrane. **B:** VPA-FITC solution reservoir. **C:** washer reservoir. **D:** reaction chamber. **E:** waster chamber. **V1, V2, V3:** capillarity-driven stop valve for the pre-loaded PBS, VPA-FITC and washer solutions respectively. **V4, V5:** capillarity-driven stop valve for the mixed fVPA, VPA-FITC solution. **V6:** Liquid-triggered microvalve which establishes flow into **D** once both liquids reach the junction. **Yellow poles:** electrodes for the electrolysis-based injector.

3. Technological feasibility

Because of the coronavirus pandemic, the BiosensUM 2020 team did not have access to laboratories to properly prototype and test the presented concepts. To prove the technological feasibility of our ideas, we rely on our rigorously conducted literature research and our precise and comprehensive calculations. Most potential problems with our concept that could be solved in the lab are acknowledged in this document and will be addressed as soon as the situation permits it.

Affinity is the strength of binding of a single molecule to its ligand. It is typically measured and reported by the equilibrium dissociation constant (K_D), which is the ratio between the antibody dissociation rate (k_{off}), and the antibody association rate (k_{on}). For an optimal competitive assay, the fluorescent and unmodified molecules should have a similar affinity for the antibody. Otherwise, the equilibrium could be drastically shifted and result in a competitive assay in which one of the competitors occupies most of the binding sites. The K_D value of VPA and its antibody is known to be about 625 μM,[5] but we can also experimentally assess the relative affinity of the fluorescent derivative and original molecule with the antibody by using surface plasmon resonance (SPR), once we regain access to the laboratories. The fluorescent derivative comprises a fluorescein moiety which could cause steric hindrance, thus disrupting the ability of other molecules to efficiently bind to neighbouring antibodies. In order to determine to what extent the presence of this large tag can modulate the affinity of the fluorescent derivative for the antibody relative to the analytical molecule, it will be necessary to perform tests involving SPR methods for both VPA/antibody and VPA-FITC/antibody couples, and then calculate their respective K_D. To better understand this phenomenon, experimental studies on the effect of a molecular linker between the target molecule structure and FITC moiety could also be conducted, as results reported in the literature on the subject are rather conflicting.[6,7] Such studies could be achieved by using linkers of different lengths to determine its effect on affinity.

Although the pandemic did not allow us to collect the experimental data required to mathematically describe our competitive assay using comprehensive equations, previous studies aimed at the development of immunoassays involving either VPA or other common anticonvulsant drugs and a fluorescein-based tag attested that reaction times of 5 minutes were sufficient to afford quantitative and reliable results.[3,8,9] We believe that the large contact area between the functionalized surface and homogenized reaction mixture, coupled with an effective spread of the incoming liquid, will accelerate the binding of fVPA and VPA-FITC to the antibodies. According to the Gibbs free energy equation, other strategies to speed up the reaction process would be to heat up the reaction mixture to a moderate temperature, at which the antibodies still preserve an appropriate conformation for competitive binding.[10] We are thus confident that a reaction time of ∼3 minutes would be sufficient for the assay to take place quantitatively.

Since the competitive assay is based on a specific interaction between the free analyte and the antibody, the behavior of the analyte does not depend on the surrounding constituents in the matrix. Thus, external calibration will be adequate. Typically, between 5 and 10 solutions containing various known concentrations of analyte and a constant concentration of fluorescent derivative are prepared for proper calibration.[11] The suggested concentrations to perform the calibration curve for VPA and CBZ are given in the table 2 presented in the Appendix D. It would be important to validate that the variation of fluorescence as a function of concentration is important over this range of concentrations to confirm the appropriate sensitivity of our assay. Moreover, several controls will be included in order to ensure the sustainability of the results by having a control below the therapeutic range, one in the therapeutic range and one over the therapeutic range (see table 3 in appendix D). Once the calibration curve is determined, it is possible to calculate the limit of detection (LOD) and the limit of quantification (LOQ). Also, other sensing parameters such as the accuracy (R^2), the standard deviation (SD), and the variance can be determined.

In order to keep the monoclonal antibodies immobilized on the cartridge for a long time for the purpose of the commercialization, the lyophilization method, which consists of drying the antibodies at a very low temperature, is used to preserve the integrity of the molecules and to reduce damages (Appendix E).[13,14] The freeze-drying process occurs in three stages. The freezing consists of cooling the surface (by mechanical refrigeration, liquid nitrogen or dry ice). The drying consists of pumping the air out the chamber while a small amount of heat causes the ice to turn into water vapor. The material dries out gradually, and it is then sealed in a moisture-free package (with a silica desiccant bag inside to keep the humidity low).[15] Once lyophilization has been carried out, the antibodies can be stored at -20 °C for 3 to 5 years without losing any activity and a few months at room temperature.[13]

The biomedical engineering side of the project relies on the physical concepts of diffusion, surface energy and electrolysis. Our biosensor utilises microdialysis in order to separate fVPA from the protein-bound VPA in our blood sample. A 10µm thick microporous membrane with 20% porosity and pores of radius 0.2µm is used, allowing the fast migration of fVPA molecules. The diffusion coefficient of valproate has been empirically established at $6.52\times10^{-6}cm^2/s$. Because VPA's hydrodynamic radius (0.353nm) is far smaller than the size of the pores, Renkin's equation predicts that the effective diffusion coefficient for VPA in this situation corresponds to over 99% of VPA's diffusion coefficient in bulk solution. Larger molecules such as proteins and protein-bound VPA, having far superior hydrodynamic radii (e.g. 3.55 - 5.85 nm for albumin)[16] take longer to diffuse through the microporous membrane. Our analysis relies on the biosensor's ability to let the diffusion of VPA quantitatively take place while also stopping the process as soon as possible to let a minimal number of large molecules pass through the membrane. We estimate the effective diffusion time to be ~1min45 (Appendix F) using the mathematical model described in a work by Breault-Turcot et al.,[4] where this microdialysis method was successfully implemented. This estimate takes into consideration the discrepancies between the theoretical estimates and empirical results from the original article since we have multiplied by a "correction constant" of 300. This ensures that we are not underestimating the diffusion time and supports the feasibility of our concept.

Our carefully designed microfluidic channels take advantage of the capillary forces that act on micron-scale channels. Specifically, we can rely on the surface energy of our system at specific points on our chip to ensure that pressure barriers control the fluids' flow. For instance, when a small channel (in our case 5µm of width) suddenly widens into a larger channel, the liquid stops at the enlargement.[17,18] This phenomenon is mathematically modeled by the total interfacial (surface) energy of the system which is a function of solid-liquid, solid-air and liquid-air interface areas and the surface energy per unit area coefficients (respectively 38.5, 19.8 and 72.2 mJ/m^2)[19] specific to the PDMS/PBS/Air trio. Using Young's equation and the derivative of the surface energy with respect to the volume of injected liquid, we determined that by using angles of enlargement of ~90° and a doubling of the channel's width we can ensure that the capillarity-driven stop valves will effectively stop the flow when desired. This method has been successfully used before.[17] For the detailed calculations please refer to Appendix G.

When the flow of liquid needs to be re-established, it is sufficient to increase the pressure applied on the stopped liquid until the pressure barrier at the channel's enlargement is broken. In our concept, the enlargement located barriers are broken by using an electrolytically-generated O_2 bubble created at a cathode inserted into the chip's microfluidic channels, the corresponding anode being placed further back in the fluid's flow. This method is several orders of magnitude more efficient than other methods used to break the pressure barrier such as expansion of the liquid through heating.[17] Previous empirical results[17] ensure us that regardless of the O_2 bubble's expansion in the microfluidic channel, the liquid flow is not completely impeded, thanks to the presence of capillary-induced "creep" flow along the channel sidewalls and around the bubble's edge.

A liquid-triggered liquid microvalve is used to mix the fVPA PBS solution with the pre-load VPA-FITC solution and to lead their flow towards the reaction chamber. The design is inspired from an article [18] where it was successfully implemented, but the original design was modified to accommodate the mixing of different volumes of liquid by making the two openings proportional to the volume of liquid passing through each of them. Different geometries will be tested in the lab to ensure that the valves operate properly. The dimensions of our microfluidic cartridge and the volume of the pre-loaded liquids (0.13µL, 5µL, 10µL respectively for the PBS, VPA-FITC and the washer solutions) have been specifically selected in order to accommodate the full testing range for total VPA concentration: 10-150µg/mL. They ensure that the concentration of fVPA in the reaction chamber varies between 0.1µg/mL and 2.1µg/mL, which corresponds to a range found in the literature for a FITC-based competitive immunoassay. The chosen microcontroller is easily programmable and compatible with all chosen electronic components. Moreover, the technical part of the fluorescent immunoassay is well studied and straightforward to implement. The implementation of powerful data analysis algorithms together with our precisely generated calibration datasets/curves will allow us to provide precise results. All the design choices of our cartridge and our biosensor have been thoroughly justified by empirical results from the literature and extensive calculations evaluating their feasibility. We are confident that our concept has the potential to achieve the required analytical performance in under 5 minutes since the competitive assay will last about 3 minutes and the dialysis 1min40 or less according to our estimates. All the other steps of the testing process are done almost instantaneously as they mainly involve fluid movement over short distances.

4.Originality

The development of our concept was guided by the ambition to offer an all-in-one sensor, requiring no sample pre-treatment and effectively delivering quantitative results in a matter of minutes. Although the microdialysis concept was one of the potential way to recover fVPA from blood samples proposed in discussions with our team supervisor, Prof. Jean-François Masson, the selection and ensuing adjustments to the concept were made by the team members to ensure the consistency of this separation step with respect to our fluorescence assay and the time constraints established by SensUs regarding the analysis. To the best of our knowledge, no fluorescent assay currently reported involves such an all-in-one separation step. In this context, our team quickly understood that the dimensions of the microdialysis chambers and the surface area of the porous membrane were parameters which, when strategically adjusted by means of mathematical calculations, could help reduce significantly the separation time. To provide realistic results, the team not only used theoretical formulas, but also decided to apply a correction factor, whose exact value was determined according to reported discrepancies between theoretical and experimental results, to account for the passive nature of the diffusion step involved in our concept. It is worth noting that in recent years, no graduate researcher has worked on this microdialysis concept at our hometown university (Université de Montréal) since Breault-Turcot in 2015.[4] Therefore, all calculations and adaptations of the concept have been performed solely by BiosensUM team members.

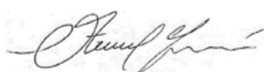
The team also realized that the introduction of the VPA-FITC derivative had to be completed after the microdialysis step, only in the presence of fVPA, so that the proportion of unbound VPA relative to the protein-bound VPA would not be affected by an equilibrium change induced by the presence of VPA-FITC in the whole blood. As such, although no active member of our team had major knowledge in microfluidics, we were able to conceptualize our cartridge and assess its potential efficiency independently, in order to meet, to the best of our ability, the requirements associated with our fluorescence assay. Through in-depth literature research,[5,18] we harnessed the theory associated with microfluidics to apply the pertinent concepts required to fulfill our needs, which were, from a technical point of view, mainly to limit the passage of blood proteins through the porous membrane, ensure the combination and simultaneous inflow of the analyte and the VPA-FITC derivative in the reaction chamber, and guarantee an appropriate washing of the latter prior to fluorescence measurements, all of this in a continuous, successive and coherent fashion. This way, we agreed on implementing electrolysis steps,[5] channels of specific geometries,[5,18] and passive liquid-triggered valves[18] to precisely regulate the flow of the various liquids in our cartridge. While these concepts are inspired by academic work which we independently uncovered, they have been modified to a large extent and adapted to our own situation, sharply different from the theoretical context in which they were first introduced in the literature. The integration of these components has thus been thoroughly verified and validated by comprehensive calculations carried out by team members.

Along the way, our supervisor and the consultants from the TTP firm (SensUs partner) occasionally helped us to validate the proposed concept while giving us advice for the next steps. By taking advantage of each team member's strengths, we have thus succeeded in setting up what we believe to be an original and versatile system, whose operation does not rely on complex mechanics, aiming to solve a real problem in a simple way. It is with the same intention that we independently concluded, following additional calculations and stimulating discussions with a wealth of health professionals, that our biosensor would position itself as a novel system able to effectively monitor several anticonvulsants.

Team supervisor's statement

This year's team Canada showed an unprecedented level of autonomy. I certify that they worked independently from any researcher at our institution and that this project does not emerge from another research project. As such, I confirm that the ideas and innovations are solely from the participants. Early in the contest year, I have exposed them to the theory of various biosensing platforms, to multiple ways of separating proteins from small molecules and various biosensing schemes or types of immunoassays. The decision on the choice of technology was theirs, I only validated its viability. In addition, my personal involvement with the team was more limited than other years. At the onset of the pandemic, I was mandated to build a new biosafety level 2 lab on a different campus, recruited and trained researchers for a COVID-19 project and became heavily involved in science communication in the national media. It meant that they were essentially on their own for the critical period of time between March and June. To my delight, they had already solved most of the challenges when I reconnected. I only addressed their questions and gave them a quick tutorial on business development.

Prof. Jean-François Masson
Team Supervisor



Arnaud Laramée
Team Captain

Asmae Danouj
Team Captain

5. Translation potential

5.1. Business model canvas

Problem
Time consuming → blood sample taken by a nurse + multiple steps required for purification

Heavy process → Laboratories and heavy equipment needed → Inaccessible in remote areas

Existing Alternatives
Tests conducted in laboratories (determination of anti-seizure drugs levels in blood): EMIT, CEDIA, FPIA

Solution
Easy-to-use portable biosensor providing clear test results in minutes in a one-step process

Technology adaptable for several molecules: one biosensor → dosage of several molecules according to the cartridge

Key Metrics
Key actions: Use of the sensor by health-care professionals: Sales, # distributors
Success metric:
15-20% of market

Unique Value Proposition
No large blood sample and no purification → ≠ nurse ≠ lab tech ≠ equipment → Doctor's autonomy

Reduces the congestion of health care centers

Makes the tracking of anticonvulsants accessible locally for small communities

Unfair Advantage
Tracking of multiple molecules with the same biosensor

Portable device with a one step process

Patent

Channels
Conferences/events

Contacts in Pharmaceutical compagnies

Health researchers: publishing papers

Customer Segments
Ministry of Health and hospitals (customer)

Physicians (e.g., neurologists and psychiatrist) (Users)

Nurses and caregivers (users)

Early adopters
Physicians (e.g., neurologists) in remote areas

Clinical biochemists

Cost Structure
First five years:
Production – 755 460 $ CAD
Marketing – 168 000 $ CAD
R & D – 578 000$ CAD
Administration – 225 960$ CAD
Human ressources – 2 264 000$ CAD

Revenue Streams
Sale of biosensors and cartridges to government and health care centers : 0,62M $ cartridge and 3.5M $ biosensor
According to our current estimations, we plan on selling around 700 biosensors and 31 000 cartridges to healthcare centers all over Canada during the first five years. This would generate a gross revenue of 4,12 million canadian dollars.

5.2. Stakeholder desirability

Epilepsy is a common neurological disorder treated with anticonvulsants for 70% of patients. Valproic acid has emerged an effective option to manage this condition and is now broadly used, although not all patients have the same response to this drug. Blood samples are taken every 3-12 months to measure its free concentration in blood, since it can be impacted by many factors in patients.[21] Currently, conventional techniques allow such measurements (Appendix H), but the large volume of blood needed combined with the tedious purification steps, harsh reagents and heavy equipment involved makes the expertise of nurses and laboratory technicians a requirement and is time-consuming.[22, 23]

We offer an easy-to-use portable biosensor that provides clear test results in a matter of minutes for optimized and personalized treatment through anticonvulsants monitoring in blood. The sensor is based on a competitive fluorescence assay, which allows to readily monitor VPA as well as other common anticonvulsants, Carbamazepine (CBZ) and Phenytoin (PHT), according to the exact interchangeable chip being used. Our sensor most notably stands out from other technologies by its speed and ease of use (Appendix H). The physician can get the result autonomously in the office, in minutes, without further lab testing. Less thant 1 uL of blood obtained by a lancing device is needed, and the whole blood can be injected in the device without any prior purification. Our solution thus gives a direct evaluation of the free anticonvulsant fraction though a one-step, point-of-care process. After a few minutes, the results are displayed on a digital screen and the clinician can readily add them to the patient record. In an ulterior generation of our sensor, we plan to implement a software that would automatically execute the data transfer, as this commodity was regarded as highly relevant by professionals.

Most doctors we interviewed mentioned that there is a limited need for a portable device limited to the sensing of a single anticonvulsant, especially in urban centers, where laboratories are often adjacent to healthcare facilities. Therefore, we came up with a concept shaped according to tangible needs. The ability to measure the levels of multiple target analytes appeared as a valuable benefit. CBZ, which is used for the treatment of epilepsy as well as in psychiatry just like VPA, is the second target we selected. We also plan to quickly consider another molecule: PHT. Ultimately, we aim at being able to test through a single device all common anticonvulsants suitable for a fluorescence-based competitive immunoassay, an idea largely validated by professionals. In the first generation of our sensor, a specific cartridge has to be inserted for each target molecule. However, for the second generation, we plan to develop a multiplex test: with a single cartridge and a single test, the simultaneous quantification of multiple anticonvulsants will be achieved. It is thus worth emphasizing that our technology allows for the

formulation of tests for a wealth of analytes by making only simple modifications to the inserted cartridge, making the device suitable in numerous contexts. Another targeted need emerged while considering the population distribution in Canada: more than 30% of Canadians reside in remote areas throughout the vast territory and in several cases, far away from hospitals equipped with a laboratory. Thus, a major challenge for the government of Canada is to make health care readily accessible to all, especially when heavy and expensive equipment is required. In this context, a biosensor can help make certain tests accessible and reduce the costs associated with the shipping, handling, and preservation of crude blood samples. Pediatricians and psychiatrists could also be highly interested in our product, as their patients, kids and psychiatric patients, respectively, often have an indisposition towards blood tests and needles, which can make the traditional blood tests harder to perform and therefore more expensive. Furthermore, these groups of patients are more prone to deviate from the appropriate posology, and kids require more frequent dose adjustments due to their rapid growth and development, which suggest that our technology is particularly attractive to pediatric and psychiatric units, which could represent an important customer segment.

In Canada there is a federal legislation, *Canada Health Act (CHA)*, for publicly funded health services. Most physicians are self-employed and paid by the state on a fee-for-service basis, and medical equipment costs are assumed by the Ministry of Health.[24] Physicians first express the need for a specific technology and the Clinical Biochemistry Department can proceed with a tender and purchase the technology. This creates a unique environment where the applicant does not bear the cost. Doctors do not consider the price of the technology; they consider the gain in their medical practice. Managers must decide based on the strength of the need expressed by the physicians and the cost of the technology relative to current techniques.

5.3. Business feasibility

The first three years are considered as development years and the fourth and fifth years, as production years. Therefore, in the early years of our startup, we will evolve in one of the great local business incubators such as Quebec Center for Innovation in Biotechnology (CQIB), District 3, CenTech (in the top 20 world incubators). In these incubators, the premises and the necessary equipment are available, allowing us to manufacture the prototypes on site at reduced costs. If needed, especially for the microfluidic aspect, companies like Moore Med.Tech, Nxtens Microsystems Inc, TransBIOTech will be consulted. We plan to secure a partnership with a monoclonal antibody manufacturer and an optics company since they are two main components of our technology: it could lead to a considerable production cost reduction. An expansion into the US market is planned for the sixth year of our startup, once it will be well established in Canada. It will most likely be a challenge as the US health care system has a different structure and relies on private insurance companies. We will need to secure strong partnerships with local distributors who have an already well-established and anchored network. By joining our technical knowledge and technology to their network, both parties can benefit from this partnership through sales.

Literature and interviews revealed that the biotechnology market is very challenging for startups, as they enter a competition involving large pharmaceutical companies.[25-27] In Canada, four major companies make most medical equipment sales: Abbott Laboratories, Roche Diagnostics, Siemens and Beckman Coulter. Therefore, we selected a B2B2C marketing model: the product will be sold to a pharmaceutical that will then sell it to doctors and hospitals. The pharmaceutical company can make a profit without having to spend on the technological development, and simply take advantage of its network to do the marketing. It is advantageous for the startup, which doesn't need to do as much marketing. The chosen channels should add value to the customer and improve their experience as users. In our case, doctors are busy and seek simplicity. There is therefore a strong advantage to doing business with companies that already have representatives in contact with health professionals. In addition, since we are selling a product used in the clinical field, confidence in results and credibility are two important factors. Doing business with an already reputable company can increase doctors' confidence in the technology. Papers will be published to promote the accuracy and efficiency of the biosensor, and its promotion will be underscored in conferences, among others.

The startup will consist of three teams: science, technology and business/administration (Appendix I). The science team is composed of chemists and biochemists able to synthesize the molecules and carry out the required experiments for molecular detection. The technology team will be composed of biomedical engineers to design the cartridge and the chip by integrating fluid management, optical and electrical path. A computer science engineer will oversee data management and interface. A person trained in business will aim at creating lasting relationships with the strategic partners listed above, raising funds managing the day-to-day operations of the startup. The science and technology teams will work together during the first two years to prototype the first generation of biosensor and chips, generate experimental results and publish papers (Appendix J). During the first two years of development, the business team will oversee securing a partnership with a pharmaceutical company for a B2B2C business model and will apply for a corporation status, and later secure partnerships with manufacturers. The three

teams will collaborate in the first two years to raise funding, apply for a patent and get an *Investigational testing study permission* delivered by Health Canada (Appendix K).[28] Once the authorization is obtained, the science and technology teams will work together to conduct clinical trials and obtain *Marketing Authorization* delivered by Health Canada. By the end of year three, the team will be able to expand by including junior chemists and engineers and machinists who will support the production while senior scientists will oversee R&D to develop cartridges for other molecules in a multiplex assay as well as an optimized software. We will also seek consulting services from an accountant for book-keeping, and a patent agent for intellectual property.

5.4. Financial Feasibility

VPA and CBZ are pharmaceutical molecules part of the anticonvulsant family. During 2018, in Quebec (a province of Canada), approximately 168 745 people were prescribed anticonvulsants, including VPA and CBZ.[29] The market size in Quebec for these two drugs is about 32 736 people.[29] Patients get tested 1 or 2 times a year, making the number of tests close to 65 472/year in Quebec for these two molecules. Considering that Quebec represents 8.5 million of the 37.5 million Canadian population, we calculate that approximately 288 847 tests per year are performed in Canada, in which there is about 1162 hospitals and approximately 10 000-15 000 clinics.[30] We estimate that approximately 12 000 biosensors can be sold in Canada. No sales are expected in the first two developmental years. For the third year, we expect to sell 150 biosensors and 5000 cartridges to clinics (~1% of market), for the fourth year, we expect to sell 250 biosensors and 9000 cartridges (~3%), and for the fifth year, we expect to sell 300 biosensors and 17 000 cartridges (~5%).

Currently, the cost per test for VPA and CBZ monitoring is 5.40 and 8.40$, respectively, according to the Ministry of Health in Quebec (financial year 2019-2020).[31] However, the cost does not include that related to the collection and transport of the blood sample, and the cost of the associated equipment and its service contract. By adding these collateral costs, the total costs are 21.00-35.30$ for VPA and 24.00-38.30$ for CBZ. To estimate the cost of production, a powerful model was used (Appendix L). It can be used to estimate the cost of cartridges and the cost of biosensors themselves, as a function of the number of units produced. It is also comprised of a large number of tunable parameters, which allow the overall cost to be assessed according to various business models, expansion rates, and societal contexts. The model allows us to estimate a production cost of 7.71-10.28$ for the cartridge and a production cost of 261.09-455.60$ for the biosensor, depending on these parameters. The biosensor will be sold to pharmaceutical companies for 5000$ and they will sell them to health care facilities with a profit margin of 20-30%, which leads to a price between 6000-6500$. The cartridges will be sold to pharmaceutical companies 20$ and they will sell them for 25.00-28.57$. This brings us to a competitive price compared to current tests and allows us to focus on the simplicity gain brought by our technology.

Our local market offers a significant advantage: compelling financing. The province of Quebec is trying to position itself globally in the biotechnology market, which results in an enormous availability of public and private funding (Appendix M). We expect to be able to raise at least $1 million. This funding will support prototyping, R&D, and all administrative and human resource costs during the first two years, before our startup reaches profitability by the third year onwards. During the fifth year, further investment rounds will be made in order to extend to the United States, and grow our company. This global profile allows us to obtain a financial plan for the first five years. More details in Appendix N.

Table 1: BiosensUM financial statement 2021-2025

Year	2021	2022	2023	2024	2025
Costs	559 152	555 490	742 250	940 289	1 194 240
HR	357 760	368 492	415 947	511 626	610 175
G&A	46 868	32 473	32 473	57 073	57 073
R&D	103 600	103 600	113 600	123 600	133 600
COGs	20 924	20 924	147 229	211 990	354 392
Marketing	30 000	30 000	33 000	36 000	39 000
Revenue	575 000	685 000	850 000	1 430 000	1 840 000
Financing	575 000	685 000	0	0	0
Sales	0	0	850 000	1 430 000	1 840 000
Gross profit	15 848	129 510	107 750	489 710	645 759
Net profit (-20,6% taxes)	15 848	129 510	85 553	388 830	512 733

6. Team and support

Contributions of the Team Members

Asmae Danouj (Team captain): Asmae has played a major role regarding team management and cohesion. She was deeply involved in perfecting the concept through microfluidics ideation and calculations, as well as cartridge and device modelization. She also contributed to our market study by planning meetings with health professionals.

Arnaud Laramée (Team captain): Arnaud has maintained a primary role regarding team management and progress tracking. He actively took part in the conceptualization and verification of the microdialysis system, assay, and business plan.

Katia Hitache (Business team leader): Katia has greatly contributed to our market study through the organization of meetings with professionals and literature review,and has played a preeminent role in putting together the translation potential section of our TRD. She has also taken part in several scientific and technological aspects of the project.

Maryam Hojjat Jodaylami (Science team leader): Maryam has put great effort into coordinating the activities of the science team, and was actively involved in completing the TRD.

Stefan Horoi (Techno team leader): Stefan was deeply involved in developing the fundamentals of our microfluidics and perfecting the microdialysis process through calculations. He also greatly contributed to our understanding of the optics and signal processing, as well as the writing of the technological aspects of the TRD. He was also involved in the business part.

Lucia Gabrielli: Lucia took part in establishing our competitive assay and understanding the associated variables, while also being involved in the production, edition, and revision of the TRD.

Aaron Gabriel Nunez Avila: Aaron conducted a thorough literature research in order to establish the optimum synthetic pathways associated with the fluorescent derivatives used in our assay, while also being involved in the production, edition, and revision of the TRD.

Joseph Goldgewicht: Joseph gave us introductory pieces of advice regarding our microfluidics and signal treatment. He did not take part in the production of the TRD.

Sandrine Nicolas: Sandrine participated in finding our protocol for antibody immobilization and understanding the variables associated with functionalized surface preservation, while also being involved in the production, edition, revision of the TRD.

Ouardia Touag: Ouardia contributed to our market study through the organization of meetings with professionals and literature review, while also being occasionally involved in the technological aspect of our concept. She did not take part in the production of the TRD.

Yannis Tarfa: Yannis was barely involved in any part of the project.

People who have given support

Jean-François Masson (Team supervisor): Prof. Masson has introduced us to the theory behind various sensing methodologies. He was also available to address our questions throughout the duration of the project.

TTP team (SensUs partner): The scientists and engineers of TTP gave us an insight on the variables associated with the production of our cartridge and device, as well as on market expansion. **Various health professionals**: The professionals (neurologists, psychiatrist, pediatrician, pharmacists, biochemists, etc.) we approached throughout the project have helped us identifying the tangible needs of anticonvulsants monitoring.

Sponsors

The direction of Université de Montréal and ASEQ (Health and Dental Insurance for Students) generously contributed to our project through financial support.

7. Final remarks

We warmly thank our supervisor (J. F. Masson) for the support throughout the project, TTP team for the judicious advice, as well as the SensUs organization for making such an enriching experience possible. We hope to further our concept through experimental testing when access to university laboratories will be allowed here in Canada.

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9. Appendix

Appendix A: Synthesis of fluorescent derivatives

Fluorescein-functionalized derivatives for VPA, CBZ, and PHT, three widely used antiepileptic drugs, can be successfully synthesized using the following pathways and procedures.

i. Valproic acid

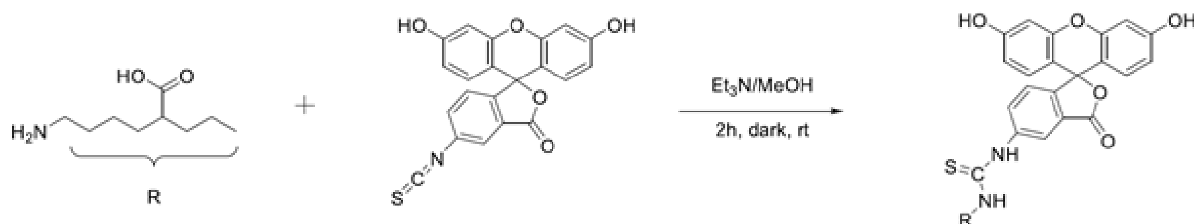


Figure 2. Scheme of VPA fluorescent derivative synthetic pathway. [2,3]

ii. Carbamazepine

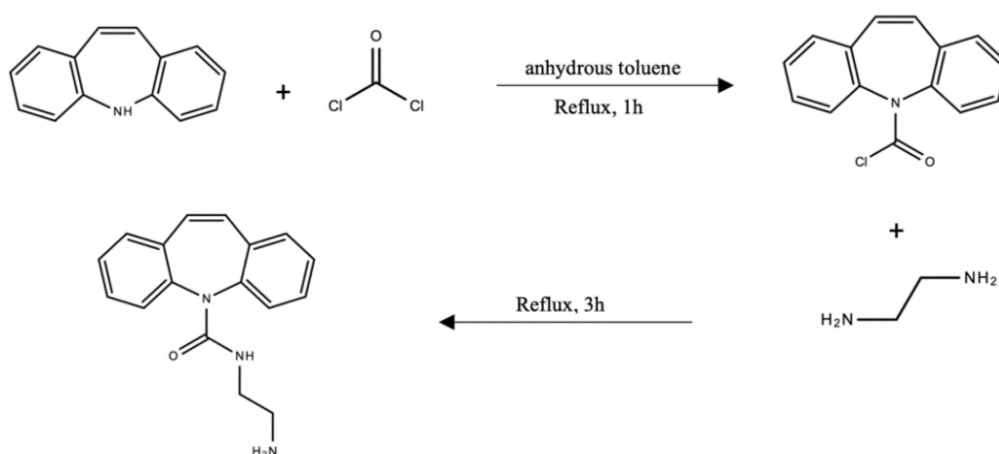


Figure 3. Scheme of CBZ derivative precursor synthetic pathway.

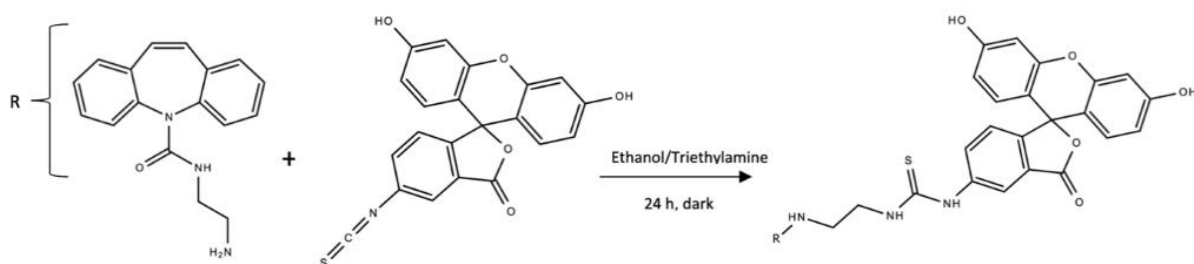


Figure 4. Scheme of CBZ fluorescent derivative synthetic pathway.

Carbamazepine-N-β-ethylamine (5-[N(2-aminoethyl)carbamoyl]-5H-dibenz[b,f]azepine, CBZ-ED, Figure 3) is prepared by dissolving 1.93 g of iminostilbene in 50 mL of anhydrous toluene and then adding this solution dropwise with stirring to 10 mL of a 110 g/L solution of phosgene in toluene. The mixture is heated under reflux for 1 h, by taking care to avoid any moisture. When the mixture reaches 0 °C, 1.3 mL of ethylenediamine is added. The mixture is reheated under reflux for 3h by avoiding the moistur. When the solution is cooled to 0 °C, a white precipitate forms. The precipitate is then removed by filtration, and the filtrate is then evaporated under reduced pressure. The residue is dissolved in chloroform and the solution is extracted with dilute hydrochloric acid. Then, the inorganic extract alkaline is made by adding concentrated ammonia. The alkaline extract is re-extracted with chloroform, is dehydrated with anhydrous Na_2SO_4, and the solvent is evaporated. The residue obtained is CBZN-β-ethylamine. [7]

To synthesize the fluorescein-functionalized derivative depicted in Figure 4, 3 mg of the CBZ-ED and 5 mg of the fluorescein isothiocyanate in 600 µL of ethanol containing triethylamine (10 mL/L) react in the dark for 24 h. Then, 200 µL of the reaction mixture is applied to a silica plate (20 x 20 cm, 1 mm thick) in order to prepare the thin-layer chromatography. The developer which is a mixture of chloroform/acetone/acetic acid (70/20/10 by vol), separates a major component (R_f 0.82). The product is then scraped from the plates, dissolved in ethanol, and stored at -20 °C. The concentration of the fluorescein labeled CBZ is estimated spectrophotometrically.[7]

iii. Phenytoin

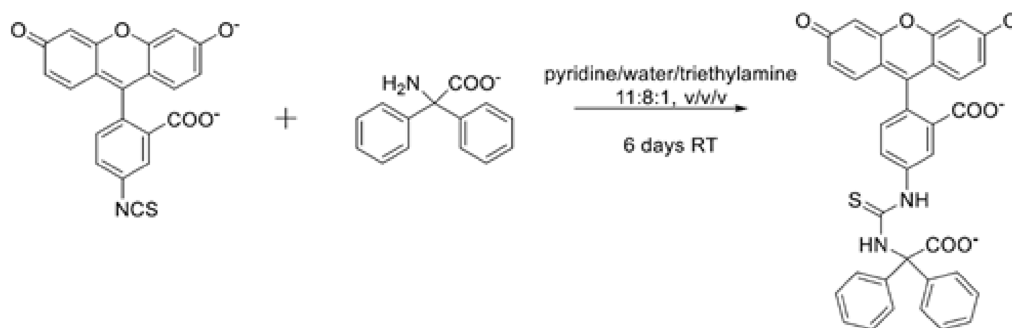


Figure 5. Scheme of PHT fluorescent derivative synthetic pathway.

Fluorescein isothiocyanate and 2,2-diphenylglycine (DPG) are each dissolved in pyridine/water/ triethylamine (11:8:1, v/v/v) and are then combined according to specific volume fractions for 6 days at room temperature. Fluorescein-containing products are then precipitated by addition of 10 volumes of 0,05 M ammonium acetate buffer, pH 4.0, and they are collected by centrifugation. The supernatant is discarded, and the precipitate is washed two times by distilled water. The precipitate is then dissolved in ammonium bicarbonate (0,05 M) with a small volume of NH_4OH (1,5 M) applied to a column of cellulose equilibrated with 0,05 M ammonium bicarbonate, adjusted to pH 9.0 with ammonia, and eluted at 6 ml/h with this same buffer. Two major components are separated, and the first to leave the column is pooled, freeze-dried, dissolved in 6 ml of distilled water with the aid of 1,5 M NH_4OH solution, and again freeze-dried. The yield of the fluorescent derivative is 20%.[32]

Appendix B: Fabrication methods

The cartridge is fabricated by soft lithography, a procedure that allows mold replication through multiple steps without specific expertise. The process begins with the production of a polymer (or elastomer)-based microfluidic chip over the antibody-functionalized glass surface. The microporous membrane can be fixed in the compartment A (shown in figure 1) to constitute the dialysis chamber. Afterwards, a thick TiPt layer is deposited on this first part of the chip to create the conductive material necessary for the electrolysis, as similarly done in a previous work.[5] These electrically conductive parts run from the interior of the microfluidic chip, where the electrolysis takes place, to the exterior of the chip where they are in contact with the biosensor's electrodes. Finally, the superior part of the chip is created, effectively closing the cartridge. PDMS will be the primary material used during prototyping. However, a more robust polymer, such as PMMA, will be used for large-scale production, mainly to reduce costs and increase robustness.

Appendix C: Exterior dimensions of the biosensor

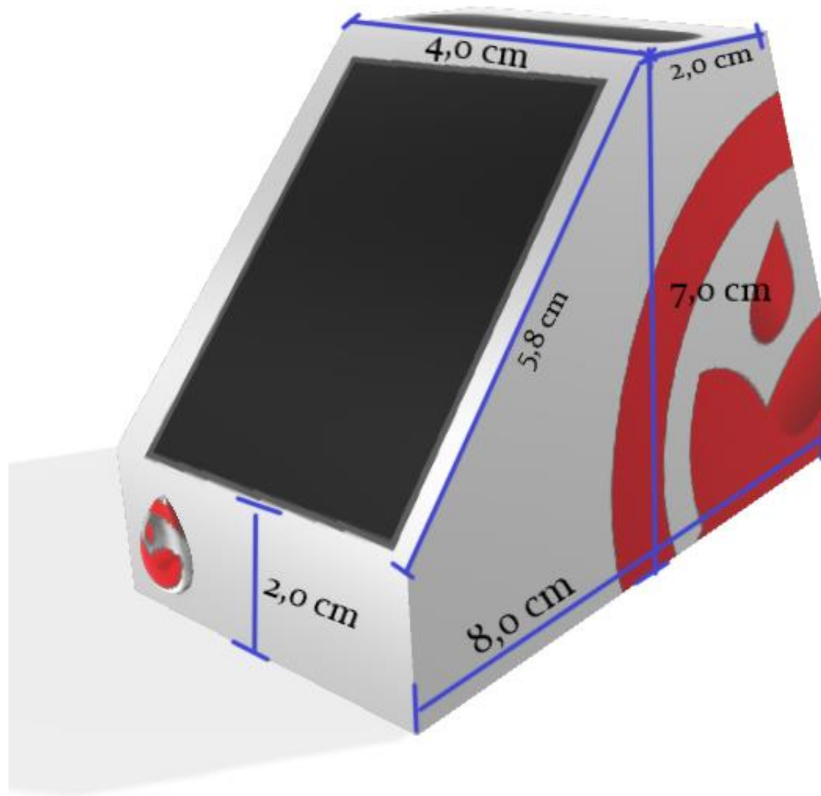


Figure 6. Biosensor design and dimensions

Appendix D: Calibration and controls

Table 2. The suggested concentrations to perform the calibration for VPA and CBZ

Valproate concentration (µg/mL)	Carbamazepine concentration (µg/mL)
0	0
0.5	0.5
1.0	1.0
3.5	1.5
6.0	2.0
8.5	2.5
11.0	3.0
13.5	3.5
16.0	4.0
20.0	5.0
25.0	6.0

Table 3. The control tests for VPA and CBZ

Control	Type	Valproate (µg/mL)	Carbamazepine	Fluorescent derivative (µM)
1	Negative	-	-	-
2	Negative	0.5	-	-
3	Negative	10.0	-	-
4	Negative	20.0	-	-
5	Negative	-	0.5	-
6	Negative	-	2.0	-
7	Negative	-	4.0	-
8	Positive	-	-	1
9	Positive	0.5	-	1
10	Positive	10.0	-	1
11	Positive	20.0	-	1
12	Positive	-	0.5	1
13	Positive	-	2.0	1
14	Positive	-	4.0	1

Specifications:

1: Ensures that the background signal without fluorescent derivative is negligible;
2 to #7: Help to ensure that valproate alone does not generate a signal even though it binds to the antibody;
#8: Ensures that the fluorescent derivative produces a signal;
#9 to #14: Ensures that a signal is produced over the full range of concentrations;
Add an additional detector to the light emitting device to correct for variations in intensity.

Appendix E: Antibody conservation

In order to conserve the antibodies immobilized on the surface, the pH of the dried antibodies must be considered. A pH of 5.0 being desirable for the IgG1 antibodies, while maintaining water concentration at a maximum of 2 to 3%.[14,33] A pH lower than this and a higher water concentration can result in aggregation of antibodies and alter their secondary and tertiary structure.[14,33] Salt buffers are required in order to control the pH.[14] To preserve the native structure of the antibodies, stabilizers must be added to the reaction, otherwise the antibodies will denature by forming β sheets.[14,33] There are different stabilizers for monoclonal antibodies, such as sugars (sucrose or trehalose) or polyols (glycerol and sorbitol).[14,33] These stabilizers have different effects, for example the combination of sugars combined with polyols prevents aggregation and addition of a small amount of sorbitol helps maintain the native structure.[33] Studies have shown that a ratio of 360 sugar: 1 protein is enough to maintain the activity of antibodies with a concentration of 3-4 folds less than the iso-osmotic concentration.[34] When water is removed during drying, stabilizers can form hydrogen bonds with proteins, thus retaining the structure of the protein.[33] Although these stabilizers are effective against dehydration stress, temperature and moisture, they are not effective against interfacial stress. Surfactant can be added as a protectant for this kind of stress, as they compete with proteins at the interface for adsorption, and they also act as humidifying agent during rehydration.[35] Some typical surfactants used are Tween80, TritonX-100, SDS and Lubrol-px.[36] To reactivate the dried antibodies, the addition of distilled water is necessary until the desired concentration is reached (PBS may be added to dilute, but distilled water is still needed at the beginning). [13]

Appendix F: Microdyalisis calculations

Variables:

- D_0: VPA diffusion coefficient in bulk solution ($6.52 \times 10^{-6} cm^2/s$)
- R_H: estimated hydrodynamic radius of a VPA molecule ($0.353nm$)
- R_P: radius of the membrane's pores ($0.2\mu m$)
- V_1: volume of liquid in the chamber situated over the microporous membrane, contains the pre-loaded PBS and the blood sample ($1\mu L$)
- V_2: volume of pre-loaded PBS in the chamber situated under the microporous membrane towards which the fVPA molecules are migrating ($1.2\mu L$)
- L: membrane thickness ($10\mu m$)
- P: porosity of the membrane (20%)
- A_m: area of the membrane between the two chambers ($2.5 \times 10^6 \mu m^2$)
- N_p: number of pores on the membrane (to be determined)
- t_{eff}: effective time of diffusion (to be determined)

The Renkin equation is used to predict the effective diffusion coefficient through à microporous membrane:

$$\frac{D_{eff}}{D_0} = (1 - \frac{R_H}{R_P})^2 (1 - 2.1\frac{R_H}{R_P} + 2.1 \times \left(\frac{R_H}{R_P}\right) - 0.95 \left(\frac{R_H}{R_P}\right)^5)$$

By isolating D_{eff} we get:

$$D_{eff} = D_0 \times (1 - \frac{R_H}{R_P})^2 (1 - 2.1\frac{R_H}{R_P} + 2.1 \times \left(\frac{R_H}{R_P}\right) - 0.95 \left(\frac{R_H}{R_P}\right)^5)$$

By the variables by their respective values we get:

$$D_{eff} \approx 6.4886 cm^2/s$$

Which is over 99.5% of D_0 the diffusion coefficient in bulk condition. Furthermore, the effective diffusion time can be written as a function of the effective diffusion coefficient and

the membrane's characteristics:

$$t_{eff} = \frac{L}{D_{effp} \times \pi_P^2(\frac{1}{V_1} + \frac{1}{V_2})}$$

We can find the number of pores by multiplying the area of the membrane by its porosity and dividing by the area of a pore:

$$\begin{aligned} N_p &= \frac{A_m \times 0.2}{\pi R_P^2} \\ &\approx 3.9 \times 10^7 \text{ pores} \end{aligned}$$

By using all found values we get that:

$$t_{eff} \approx 0.33s$$

This seems like a very fast effective diffusion time, however in the article which described this method a discrepancy was found between theoretical estimates and empirical results. For small volumes, the empirical results were about 300 times bigger than the theoretical estimates. If we multiply our estimate by 300 we obtain a more realistic estimate for the efficient diffusion time:

$$\begin{aligned} t'_{eff} &\approx 0.3302s \times 300 \\ &\approx 100s \\ &\approx 1 \text{ minute and 40 seconds} \end{aligned}$$

Appendix G: Capillary stop-valves calculations

This appendix presents the calculations ensuring that the capillarity-driven stop valves function properly.

- U_T: total interfacial (surface) energy of the system
- A_{sl}, A_{sa}, A_{la}: respectively solid-liquid, solid-air and liquid-air interface areas ()
- $\gamma_{sl}, \gamma_{sa}, \gamma_{la}$: respectively solid-liquid, solid-air and liquid-air surface energies per unit area, specific to the PDMS, PBS, air trio (38.5, 19.8 and 72.2 mJ/m^2) /item θ_c:

$$U_T = A_{sl}\gamma_{sl} + A_{sa}\gamma_{sa} + A_{la}\gamma_{la}$$

This quantity is linked to the equilibrium contact angle by Young's equation:

$$\gamma_{sa} = \gamma_{sl} + \gamma_{la}\cos\theta_c$$

By combining both formulas we have:

$$U_T = (A_sl + A_sa)\gamma_{sa} - A_{sl} \times \gamma_{la}\cos\theta_c + A_{la}\gamma_{la}$$

The total energy changes as the injected volume V_l increases and so does the pressure:

$$\begin{aligned} P &= -\frac{dU_T}{dV_l} \\ &= \gamma_{la}\left(\cos\theta_c\frac{dA_{sl}}{dV_l} - \frac{dA_{la}}{dVl}\right) \end{aligned}$$

Assuming a cylindrical liquid front and given that β is the angle at which the microfluidic channels are enlarged and α is the angle describing the shape of the liquid meniscus then we can rewrite the pressure as:

$$P = \frac{\gamma_{la}}{R \times \left(\sin\beta\cos\beta - \left(\frac{\sin\beta}{\sin\alpha}\right)^2(\alpha - \sin\alpha\cos\alpha)\right)}\left(\cos\theta_c - \frac{\alpha\sin\beta}{\sin\alpha}\right)$$

Using an enlargement angle of $\beta = 90°$ and an empirical contact angle between PBS and PDMS found in the literature of $\theta_c = 105° = 1.83rad$ we can find a formula for α at the exact place of enlargement:

$$\begin{aligned} \alpha &= \frac{\pi}{2} - \beta - \theta_c \\ &= -1.83rad \end{aligned}$$

Using these values to evaluate the pressure barrier we obtain:

$$P = 27.89kPa$$

Which ensures that our passive capillarity-induced stop valve will work as planned since an increase in pressure of $P = 27.89kPa$ is required to re-establish flow. This amount of pressure is easily achieved using our electrolysis injectors.

Appendix H: Competitors and current technologies

Table 4: Comparison of competitors and BiosensUM technology for free valproate dosing

Technology	Company	Blood sample	Toxic Chemicals	Time	Heavy equipment	One step process
Competitive Fluorescence Immunoassay	BiosensUM	✗	✗	5 min	✗	✓
Multiplied Immunoassy Technic (EMIT)	Syva Dabe Behring	✓	✓	Couple hours	Spectrophotometer	✗
Cloned Enzyme Immuno Donor Assay (CEDIA)	Microgenics Corporation Roche Boehringer	✓	✓	Couple hours	Chemistry analyzer (Hitachi 911)	✗
Florescence Polarization Immuno Assay (FPIA)	Abbott Laboratories Roche Boehringer	✓	✓	Couple hours	Automated analyzer (AxSYM)	✗

There are existing techniques allowing to measure the valproic acid concentration of the blood such as the Enzyme Multiplied Immunoassy Technic (EMIT) technique developed by Syva and commercialized by Dabe Behring (ACA, Cobas Mira), the Cloned Enzyme Immuno Donor Assay (CEDIA) technique developed by Microgenics Corporation and commercialized by Roche Boehringer, as well as the Florescence Polarization Immuno Assay (FPIA) technique developed by Abbott and marketed by Abbott and Roche Boehringer.[22, 23] These methods are frequently used, they are precise and specific but still have limitations. First, 94 to 150 µL are necessary to perform the test, which is quite large and requires a blood sample performed by a nurse. [27-29] Also, these tests are sold in "kits" which contain the reagents necessary to perform the test, including sodium azide a toxic molecule. Therefore, these tests must be performed in a laboratory by technicians. .[22, 23] Indeed, these tests require several manipulation steps and dilutions are often necessary.[22, 23] There is also waiting time between the steps, so these tests are not done fast.[22, 23] The whole blood cannot be used, so the blood sample must be centrifuged for 10 minutes to obtain the blood plasma or serum.[22, 23] This means that expensive devices, like a centrifuge, are needed.[22, 23] To obtain the results a spectrophotometer may be necessary for the EMIT test, for the CEDIA test a Hitachi 911 device is necessary and for the FPIA test an AxSYM device is needed.[22, 23] Those are big and expensive devices, only present in the laboratory.

Dosing procedure with current techniques

1. Doctor's appointment: the doctor writes a request for blood tests.
2. The patient drives to a sampling center with the request to get a blood sample taken by a nurse.
3. The blood sample is sent to a laboratory (hospital) via a transporter.
4. Once the test has been conducted, the results are sent to the doctor.
5. The patient gets another appointment to adjust the prescription.

Several actors (doctor, nurse, transporter, laboratory technician) are needed. Many steps and appointments are necessary. With our technology, only the doctor is needed, and only one appointment is required for dose adjustment on site.

Appendix I: Human resources

The team will consist of 7 persons divided in the Science, Technology and Administrative & Business teams.

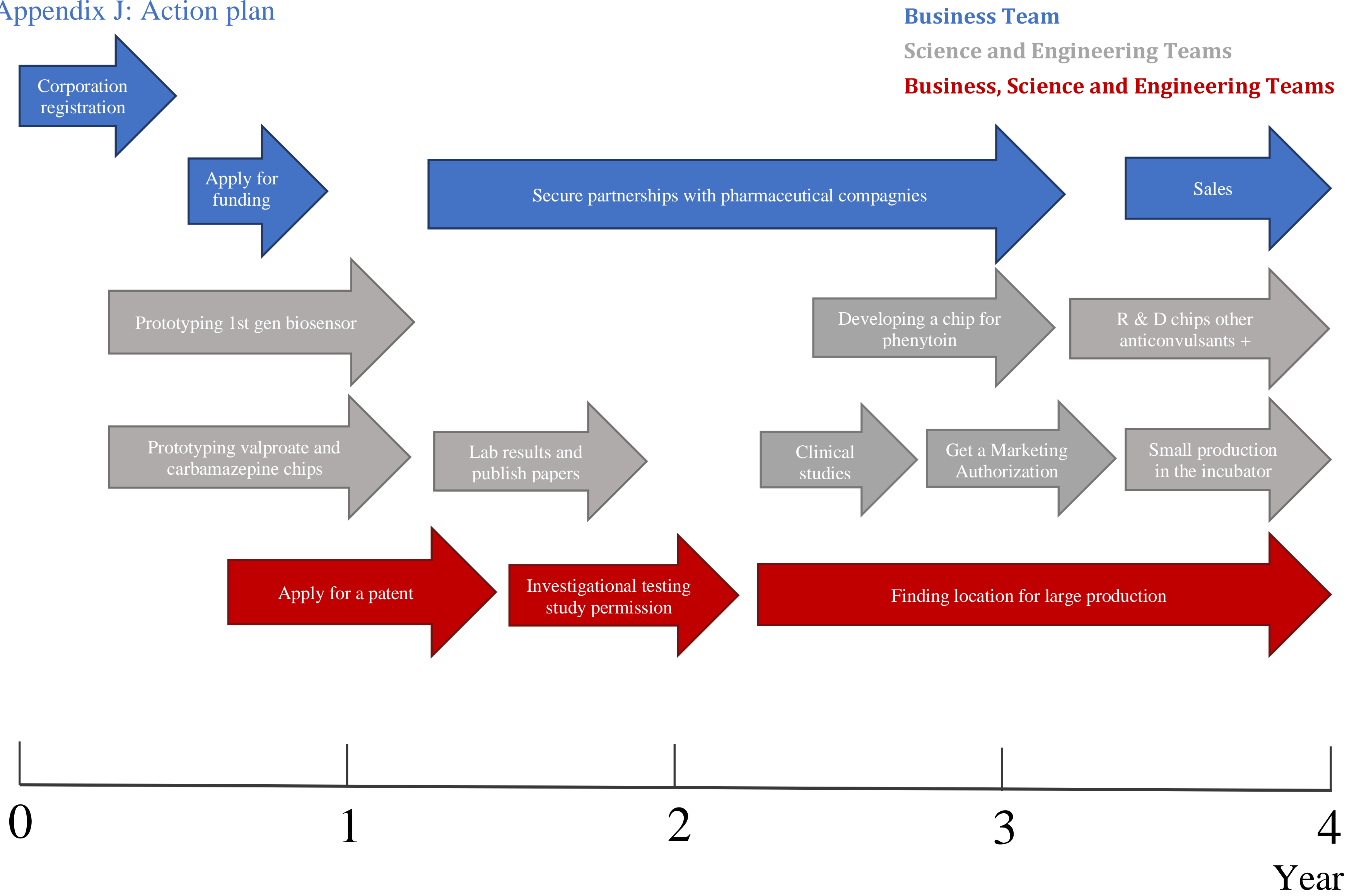
Team	Position	Responsibilities	Salary	Annual expense
R & D – Science	Chemist/biochemist Senior (Ph.D.)	Validate and develop new immunoassays	35$/h	72 800$
R & D – Science	Chemist/biochemist Junior (M. Sc)	Validate and develop new immunoassays	25$/h	52 000$
R & D – Technology	Biomedical engineer	Validate and develop more efficient microfluidics and technologies	33$/h	68 640$
R & D- Technology	Engineer (with computer science skills)	Develop software and data analysis	33$/h	68 640$
Administrative and Business	Administrative technician	Take calls and manage all paperwork	18$/h	37 440$
Administrative and Business	Business manager	Manage marketing, partnerships and legal status	28$/h	58 240$

In addition to these employees, there will be a production team added in the fourth year. The number of lab technicians and mechanics varies according to the production scale.

Team	Position	Responsibilities	Salary	Annual expense
Production	Chemist (M. Sc) – Team manager	Supervise the production of the science part of the chip	30$/h	62 400$
Production	Laboratory technician	Production of the chip	23$/h	47 840$
Production	Engineer – Team manager	Supervise the production of the microfluidics part of the chip and the box	30$/h	62 400$
Production	Mechanics/Assembler	Production of the chip	20$/h	41 600$
Administrative	Administrative technician	Take calls and manage all paperwork	20$/h	37 440$

BiosensUM

Appendix J: Action plan



Year 4

In house production and more sales

Plan USA expansion (Market study & Distributors)

Year 5

Develop multiplex assay

Plan USA expansion (Market study & Distributors)

Appendix K: Health Canada regulation

Health Canada is the federal regulator of therapeutic products, including medical devices. Health Canada reviews medical devices to assess their safety, effectiveness and quality before being authorized for sale in Canada.

Our biosensor which is a medical device that measures the concentration of drugs (in this case valproate) in the patient's blood, is a class III medical instrument, according to the Medical Device Regulation published by the Minister of Justice (section 6, part 2, rule 4f) and most precisely an in vitro diagnostic device (IVDD).[38] As indicated on the Health Canada website, the fee to pay for the examination of a class III medical device licence application is $ 7,477 as of April 1, 2020. In addition, there is an annual fee to pay for the right to sell a class II, III or IV licensed medical device which is $ 381.[28]

Investigational Testing (IT) is a study aimed at supporting the determination of safety and effectiveness of an investigational IVDD prior to general marketing. After obtaining pre-clinical data, the device is tested in the target population in Canada by the investigators to validate its performance under the conditions in which the test is intended to be used. Indeed, the following conditions should be met to obtain an authorization for IT : (a) pre-clinical analytical studies have been completed, (b) proposed cut-off between positive and negative results have been established, (c) the evidence for the safety and effectiveness of the device has not been adequately established for clinical use, (d) additional evidence for safety and effectiveness can be obtained only by trials with target populations. After the IT is obtained, we must find doctors interested in testing the device. A clinical biochemist responsible of laboratories tests explained that we need to contact the laboratory responsible also to make sure that the tested are performed by a validated alternative method also to validate the results. The data gathered during IT is reviewed by Medical Devices Bureau as evidence of safety and effectiveness for consideration of issuing a license for the device.[39] This step takes up to 75 days for class III medical devices (see the table 5).

Table 5. Service standards established by Health Canada and the Canadian medical devices industry

Service activity	Service standard for completing activity
Authorizing device license	
Class II: review time to first decision*	15 calendar days
Class III: review time to first decision	75 calendar days
Class IV: review time to first decision	90 calendar days

*A first decision can either be a decision to request additional information from the applicant or a final decision to either approve or deny the application for a medical device license.

Appendix L: Production costs

Production costs are most certainly challenging to estimate since it depends on a multitude of factors that vary greatly depending on the scale and rate of production, the materials selected, the precision of assembly, and many more variables (16-18).[39,40,41] Accordingly, it is wise to consider seeking professional guidance by meeting with regulatory agencies or specialized firms prior to production, to verify if the concept, production steps, chosen materials, and safety testing meet regulatory and clinical requirements.[40,41]

The model depicted by Figure 7and equation (1) allows to estimate the overall production cost C of a product unit (e.g., a cartridge), considering that of materials, instrumentation, equipment, rent, insurance, and labor according to a variety of production scales or, as depicted in Figure 7.

$$C = \frac{C_m}{(1-f)} + \sum_{i=1} \frac{C_{t,i}}{n}\left[Int\left(\frac{n}{n_{t,i}} + 0.51\right)\right] + \frac{1}{\dot{n}}\left(\frac{C_c}{t_{am}L} + \dot{C}_{oh}\right) \quad (1)$$

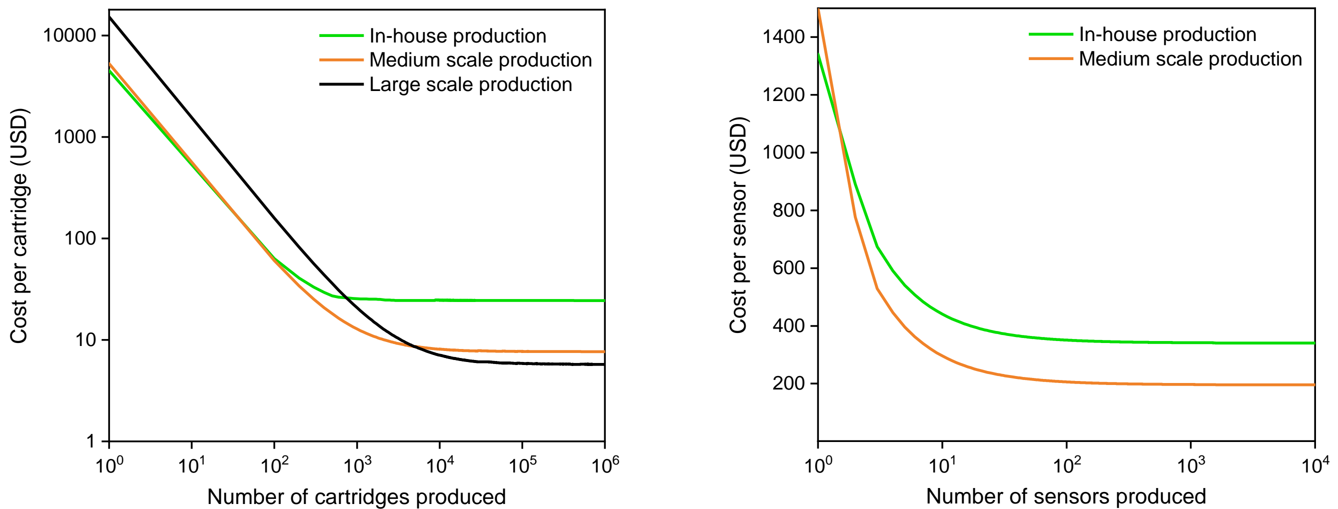


Figure 7. Costs of production of sensors and cartridges according to the number produced for three different production scales.

Appendix M: Canadian and Quebec funding

Examples of the many financing opportunities are available:

- Industrial Research Assistance Program (IRAP): up to 500 000$ - Federal grant
- Desjardins: up to 100 000$ - Loan
- Business Development Bank of Canada (BDC): Loan 7%
    - Entrepreneur of 18-39 years old : up to 60 000$
    - In the first year that we have an income: up to 100 000$
    - Many more
- Program Propulsion BioMed: 500 000$ - Loan
- Fonds de soutien à l'innovation en santé et en services sociaux : 240 000$ - Loan
- Program PME in action – Support for Capital Project Delivery (Feasibility Studies): $100,000 (Legal Analysis , Clinical Feasibility Study) - Loan
- Program PME in action – Productivity Support Component PME : 50 000$ - Loan or investor

Appendix N: Detailed financial statement

Table 6: BiosensUM detailed financial statement 2021-2025

Year		2021	2022	2023	2024	2025
Costs		559 152	555 489	705 849	819 597	986 727
HR		357 760	368 492	415 947	511 626	610 175
	Science	124 800	128 544	142 800	167 884	193 720
	Technology	137 280	141 398	171 640	239 190	308 765
	B & A	95 680	98 550	101 506	104 552	107 688
G&A		46 868	32 473	32 473	57 073	57 073
	Patent	7000	50	50	50	50
	Corporation	1500	55	55	55	55
	Accountant	3600	3600	3600	3600	3600
	Insurance	3168	3168	3168	3168	3168
	Software	20 000	20 000	20 000	20 000	20 000
	IT	9600	3600	3600	7200	7200
	Location	0	0	0	18 000	18 000
	Supplies	2000	2000	2000	5000	5000
R&D		103 600	103 600	113 600	123 600	133 600
	Experiments	100 000	100 000	110 000	120 000	130 000
	Consultation	3600	3600	3600	3600	3600
COGs		20 924	20 924	147 229	211 990	354 392
	Cartridges	4556	4556	68 340	113 900	136 680
	Biosensors	16 368	16 368	51 389	51 840	131 212
	Shipment	0	0	27500	46250	86500
Marketing		30 000	30 000	33 000	36 000	39 000
	Conferences and events	30 000	30 000	33 000	36 000	39 000
Revenue		575 000	685 000	850 000	1 430 000	1 840 000
Financing		575 000	685 000	0	0	0
Sales		0	0	850 000	1 430 000	1 840 000
	Cartridges	0	0	300 000	540 000	1 020 000
	Biosensors	0	0	750 000	1 250 000	1 500 000
Gross profit		15 848	129 510	107 750	489 710	645 759
Net profit*		15 848	129 510	85 553	388 830	512 733

*Federal and provincial taxes 20.6%