



## **Team Results Document**

# **BiosensUM**

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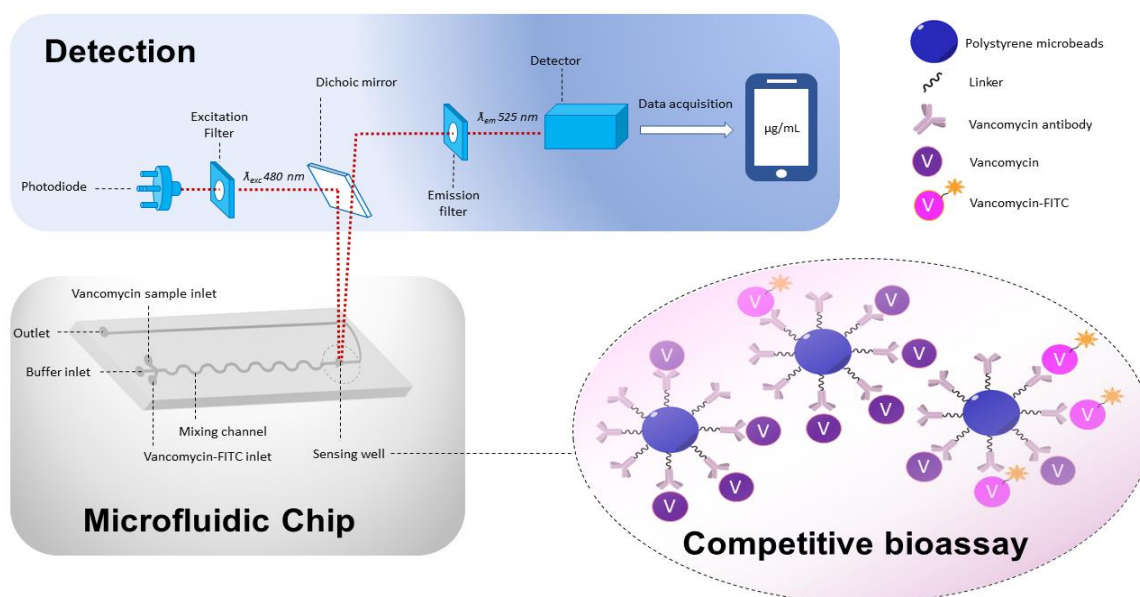
## 1. Summary

BiosensUM has designed an integrated biosensor for monitoring vancomycin concentration in blood plasma. With this biosensor, we aim to improve the vancomycin treatment procedures in hospitals by reducing the risk of under-dosing patients, which can lead to bacterial resistance, or overdosing, which can worsen the medical condition of the patient. The main component of our biosensor consists of a single-use microfluidic chip for fast, and cost-effective analysis using green chemistry principles. Our system only requires a 20  $\mu$ L drop of plasma and delivers results in less than 5 minutes, compared to the 5 mL sample tube and a minimum of 2 hours required by the labour-intensive laboratory procedures currently performed in hospitals. We have created here a portable lab-on-a-chip, which allows for the direct injection of the sample, the mixing of the reagents, the bioassay and detection of the signal to occur in a continuous flow. The signal detected is automatically converted to a working concentration value by our software application and displayed on a user-friendly LCD screen or mobile device. Furthermore, without changing the hardware or software, we can customize the chips for the detection of a variety of different molecules providing endless possibilities. We believe that portable lab-on-a-chip biosensors are the future of blood testing in hospitals and everybody from hospital management to the patients will benefit from a user-friendly and minimalistic approach such as ours.

## 2. Biosensor System and Assay

### 2.1. Competitive Bioassay

We developed a miniature biosensor based on a competitive assay for the rapid detection and quantification of vancomycin in blood plasma in a clinically relevant concentration range between 5-100 mg/L in less than 5 minutes. The biosensor consists of a microfluidic chip that requires very small volumes of reagents which allows for effective, fast and economical reactions to occur<sup>1</sup>. The unknown concentration of vancomycin in plasma will compete with our synthesized vancomycin isothiocyanate fluorescein (vancomycin-FITC) for the anti-vancomycin antibody bound on the surface of polystyrene microbeads<sup>2</sup>. The continuous flow of buffer will wash the remaining unbound molecules of vancomycin and vancomycin-FITC and a photodiode will induce the vancomycin-FITC fluorescence at a wavelength of 480 nm. Fluorescence emission is detected at 525 nm and converted to the plasma vancomycin concentration (mg/L). Therefore, as the vancomycin competes with the vancomycin-FITC, fluorescence emission will decrease as the vancomycin level in plasma will increase and it will be possible to generate a calibration curve to determine the vancomycin concentration in human blood plasma.



**Figure 1:** Development of a miniature biosensor for the detection of vancomycin in blood plasma based on a competitive assay.

### 2.2 Synthesis and characterization of vancomycin-FITC

To achieve this bioassay, we synthesized the vancomycin-FITC via the spontaneous reaction of the amine group of the vancomycin and the isothiocyanate of the isothiocyanate fluorescein in a basic solution to form an isothioureia group<sup>3</sup>. (Figure A1) The vancomycin tagged with the isothiocyanate fluorescein has been purified to 95% by liquid chromatography-mass spectrometry. (See Annexe 9.1) The mass spectrometry spectrum confirms the synthesis of the vancomycin-FITC. (Figure A2). The fluorescence spectrums of the synthesized vancomycin-FITC have been realized to find the optimal excitation (480 nm) and emission (525 nm) wavelengths in the running buffer. (Figure 2)

### 2.3 Microfluidic Chip

Technical drawing of a mechanical part, showing top and side views with dimensions.

**Top view (Scale 3:1):**

- Overall width:  $30 \pm 0.1$
- Overall length:  $62.27 \pm 0.1$
- Left end features a semi-circular profile with a radius of  $R0.5 \pm 0.1$  and a vertical dimension of  $10 \pm 0.1$ .
- Internal features include a vertical slot of width  $1 \pm 0.1$  and a horizontal slot of width  $1 \pm 0.1$ .
- Right end features a Y-shaped profile with a vertical dimension of  $1 \pm 0.1$  and a horizontal dimension of  $1 \pm 0.1$ .
- Other dimensions include  $2 \pm 0.1$ ,  $11 \pm 0.01$ ,  $10.5$ ,  $1 \pm 0.1$ ,  $1 \pm 0.01$ ,  $0.03 \pm 0.01$ , and  $0.07 \pm 0.01$ .


**Side view (Scale 3:1):**

- Overall height:  $10 \pm 0.01$
- Overall width:  $30 \pm 0.01$
- Internal features include a vertical slot of width  $1 \pm 0.1$  and a horizontal slot of width  $1 \pm 0.01$ .
- Other dimensions include  $0.03 \pm 0.01$  and  $0.07 \pm 0.01$ .

**Figure 3:** Final design of the microfluidic chip

## 2.4 Detection and Data Acquisition

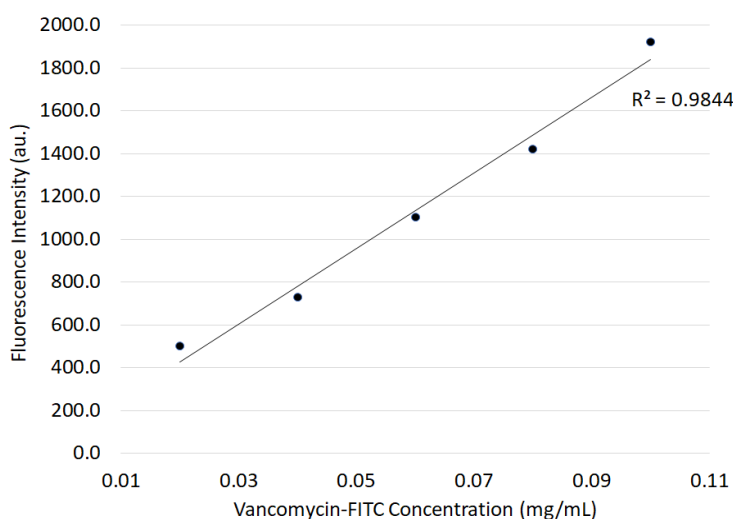
**Figure 4:** The detection and data acquisition software system (images under the creative commons copyright of Thorlabs®)



The BiosensUM logo is located at the bottom left of the page. It features the text "BiosensUM" in a blue, sans-serif font. To the left of the text is a red circular icon containing a white stylized figure of a person with arms raised, resembling a biohazard symbol or a person in a protective suit.

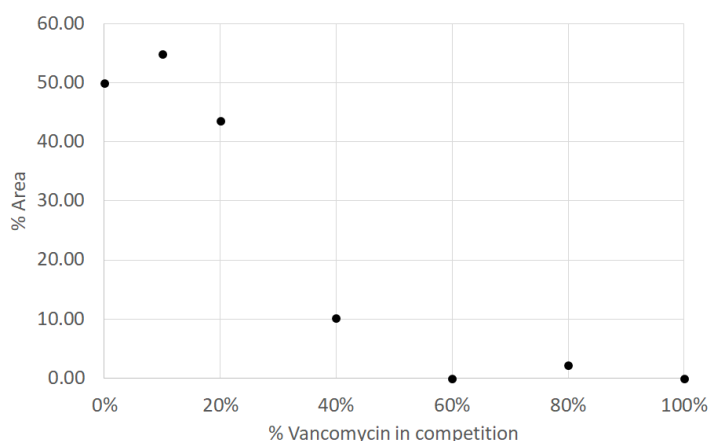
### 3. Analytical Performance

First, we tested our assay principle in a 96-wells fluorometer. We were able to detect the fluorescence of the synthesized vancomycin-FITC by this technique (Figure 2). We also performed a fluorescence calibration curve for the vancomycin-FITC linkage to our receptor, the anti-vancomycin monoclonal antibody, in the well and determined the optimal amount of



**Figure 5:** Calibration curve of the fluorescence of vancomycin-FITC bound to anti-vancomycin monoclonal antibodies (Excitation wavelength : 480 nm, Emission wavelength : 525 nm)

vancomycin-FITC that can compete with the vancomycin sample for the competitive assay (Figure 5). For the competitive assay, the tests in the 96-wells fluorometer were not conclusive. We realized that the instrument was reading the fluorescence in the solution and not at the surface of the plate where our vancomycin-FITC and vancomycin compete to bind the anti-vancomycin antibody. So, we were not able to dose the vancomycin in this condition.



**Figure 6:** Competitive assay performed by fluorescence microscopy

Then, we moved to the fluorescence microscopy to confirm our competitive assay. With this technique, we were able to do an appropriate focus on our surface and optimize the precision of vancomycin detection. With these improvements, we achieved to realize a competitive assay (Figure 6). As we expected, the lower concentration of vancomycin gave a high fluorescent coverage of the surface and the higher quantity of vancomycin resulted to a really lower fluorescent coverage. So, with fluorescence microscopy, we confirmed the principal of our competitive assay.

Thereafter, we tried to implement the competitive assay on our device. We faced a lot of obstacles because the device was not optimized enough. The major part of the problem came from the fact that the diode was not enough powerful and gave an unstable signal. Because of this, the noise was very important. And, at the end, we were not able to get a fluorescence signal significantly different of the noise one for the vancomycin-FITC. And, at the end, we were not able to perform either a fluorescent calibration curve of the vancomycin-FITC or the competition assay. To solve this problem, we had some ideas such as adding a photomultiplier and using a more powerful and stable diode.

## 4. Novelty and Creativity

### 4.1. *Already Available*

The current procedures available for measuring the vancomycin concentration in patient samples<sup>9, 10, 11</sup> are long, expensive and invasive. A registered nurse must draw a 5 mL blood sample and highly qualified professionals must perform the tests in hospital laboratories using large equipment such as a spectrophotometer and an ultracentrifuge that require daily maintenance and quality testing. This whole process can take up to 2 hours before the results are delivered to the doctor, not to mention very costly considering that a patient being treated with vancomycin needs to monitor their antibiotic levels multiple times a week<sup>9, 10, 11</sup>. This can cause errors in dosage that can be detrimental to the patient's care.

### 4.2. *New Developments*

Our goal at BiosensUM was to develop a biosensor that could improve the monitoring of vancomycin treatments in hospitals and patient follow-up. We developed a minimally invasive point-of-care biosensor that only requires a 20  $\mu$ L drop of blood and can deliver results within 5 minutes on a LCD screen or via a mobile device. A nurse or hospital personnel can perform the whole procedure in the patient's room and send the results to the doctor via our proprietary software application that can easily be paired with the existing hospital system. The treatment of the patient can therefore be adjusted in a matter of minutes preventing the dangerous risks associated with under or over dosing the patient. Our novel microfluidic system is economical, compact and environmentally friendly. The device is portable and energy efficient and the microfluidic chips only need very small volumes of reagents. The chips themselves can be altered to detect a variety of different molecules giving way to endless possibilities without having to change the rest of the hardware or software. Thanks to our biosensor, we believe we can provide hospitals with a novel approach to blood testing.

## 5. Translational Potential

### 5.1. Stakeholder Desirability

The sensor that our team developed is set to provide an excellent answer to many stakeholders' needs. First off, the patient will benefit from the faster results delivered by our device, while experiencing a more pleasant less-invasive treatment. The point-of-care feature of this biosensor allows for immediate dosage adjustments to prevent under or overdosing the patient. We also envision developing a simpler device allowing the patients to monitor their treatment at home in the same principle as a glucometer, which will significantly shorten their hospital stay.

Another stakeholder that has a lot to gain from using the apparel is the hospital management. The adoption of our product will enable them to save on labour wages by lowering the amount of work required by the current labour-intensive procedures needed to provide doctors with results. This also helps improving the efficiency of the process, reducing the necessary time from a minimum of 2 hours to a maximum of 5 minutes. Such an improvement is possible since most of the work will now be performed bed-side in the patient's room with minimal equipment and hospital personnel as well as very small volumes of sample and reagents. We foresee that all users of this device will benefit from the user-friendliness handling of this tool. However, we are still working towards being in compliance with the call-for-tender procedures in order to obtain a contract to supply hospitals with our products. A more detailed analysis can be found in our complete business plan (see Annexe 9.2).

### 5.2. Technical Feasibility

The use of a single-board computer like the Raspberry Pi (RPI) enables a myriad of possibilities, since it is a cheap and small all-purpose computer. Coupled with the potential to program with the Python programming language, it is easy to prototype quickly on the machine. Countless peripherals and other devices can be attached to the RPI, either through USB, ethernet or general-purpose input/output. By using simple scripts, the small computer controls the light-emitting diode and the motorized pump, reads the value of the photodetector using an oscilloscope, and outputs the results using an LCD screen, in the matter of a few seconds. These same scripts can be easily iterated on, improved upon and customized for other uses. The protocol that we developed to control the computer and get the results with the LCD screen or through an application could also be used by vetted partners. Our company could also offer to implement the integration of the software.

Furthermore, we put a lot of emphasis on the miniaturization of our device. The size of the digital oscilloscope was selected to be as small as possible, around the same size as the Raspberry Pi. It would be possible to substitute the latter for its even smaller, albeit slower counterpart, the Raspberry Pi Zero<sup>12</sup>. Additionally, the microfluidic chips used for testing measure only 3 cm by 8 cm. While greatly limiting blood volume withdrawn from patients, it also limits the environmental impact by reducing the quantity of reagents used in the process. It could also be possible to use eco-friendly material like degradable polymer during later stage of production.



Our future work will be aimed towards developing a custom printed circuit board as well as low-power embedded circuits that will help making it more portable, with a better battery life and at a slightly lesser cost. These improvements will be more suitable for usage in hospitals.

### **5.3. Business Viability**

#### **5.3.1 Cost projection**

In order to produce decent and reliable products, efficient cost management techniques are needed. Furthermore, funds given by other parties should be separated within the different development objectives the company is aiming for. Those objectives can be stated as: according sufficient funds to research and development, maintaining enough cash flow to sustain a short/long – term growth and acquiring the assets needed for the commercialization phase (product, marketing, patent, etc.).

For the moment, there is nobody being paid for R&D since solely team members are doing it. However, later phases might require extra work and expertise to insure the worthiness of the tester. Therefore, part of the budget needs to be directed toward research (~12K/year) and development (~20K). We gathered the cost per unit for the tester (2898,14\$), the chips (33.53\$) and the microfluidic montage (3045\$). One hospital would require a tester, a microfluidic montage and around 180 chips per month adding up to an annual cost of ~77 167.80\$. This requires us to have around 600-800K funding to have the required cash flow throughout early years. Other costs to consider: the patent (~45K), certifications (~45K) and marketing (~10K/year).

#### **5.3.2 Revenue streams**

Investment in the healthcare sector is six times higher than it was back in 2010<sup>13</sup>. This implies that an appropriate business model could generate great profit margins over the years. After reviewing different types of revenue models, a hybrid between perpetual licensing and per test billing was chosen. To drag on profit, our strategy is based on obtaining long-term contracts while still providing resources every month. Our first objective would be to gather enough funding from Canadian institutions such as the Canadian Development Bank<sup>14</sup> since those organisms can give substantial support for few costs compared to institutional capital. Then, we can start to lease the tester to as many customers as possible and sell them the chips for around five times our production cost. After that, the focus would be towards producing a variety of chips to test for different molecules, which would make us even more profit.



## 6. Team and Support

### 6.1. Contributions of the team members & People who have given us support

Name	Status	Principle contributions
Marie-Pier Dinel	Team Leader	Oversaw all aspects of the project
Madline Sauvage	Science VP.	Involved in the laboratory and performed analytical analyses
Jean-Antoine Gauthier Cyr	Technology VP.	Link between the science team and the engineering team
Zoubaire Moustaine	Engineering VP.	Microfluidic specialist
Antoine Nkaye	Marketing and Business VP.	Responsible for the entrepreneurial aspects of the project
Frédéric Fournelle	Science team member	Participated in the design of the biosensor, the development of the bioassay and the analytical tests
Elizabeth Elder	Science team member	Involved in the development of the bioassay and the scientific writing
Laurianne Gravel Tatta	Science team member	Conception of the bioassay and in the development of our analytical testing protocols
Abdelhakim Qbaich	Technology team member	Programming specialist
Godefroy Borduas	Technology team member	Participated in conceptualizing and coding the data acquisition system
Pr. Jean-François Masson	Supervisor	Helped us with the design of the bioassay and the technological aspects
Pr. Delphine Bouilly	Collaborator	Helped us with the bioassay and the detection

Finally, we would like to say a special thank you to **Vanessa Kairouz, Karine Gilbert, Antoine Caron, Jean-François Mire** and **Alexandre Bouard** without whom this project would not have been possible.

### 6.2. Sponsors

	<b>University of Montreal</b> Vice-rector of academic affairs de l'Université de Montréal Vice-rector of international affairs and of the Francophonie Faculty of Arts and Science		<b>Chemistry Department of the University of Montreal</b> Regional Centre for Mass Spectrometry
	<b>Creative Diagnostics</b>		<b>Aline Microfluidic Solutions</b>
	<b>Chemical Engineering Department of the Polytechnic School of Montreal</b>		<b>The CREATE Training Program in Continuous Flow Science</b>
	<b>The ASEQ Foundation</b>		<b>Institute for Research in Immunology and Cancer</b>
	<b>Biochemistry and Molecular Medicine Department of the University of Montreal</b>		<b>Department of Computer Science and Operations</b>

## 7. Final Remarks

The team at BiosensUM would like to thank SensUs for this amazing opportunity to participate in their international competition for the first time. It was a challenging learning experience that allowed us to grow as scientists and entrepreneurs as well as make lifelong friends along the way. We look forward to putting the finishing touches on our vancomycin biosensor and work towards acquiring a patent to pursue our project even further. We would also like to thank all our sponsors that provided us with the financial and technical support, the healthcare professionals that gave us some of their precious time to participate in our surveys and, last but not least, our two fantastic mentors Jean-François Masson and Delphine Bouilly for their guidance. We are excited for what is to come in 2019 for BiosensUM! Follow us on Instagram (@biosensum.udem) and Facebook (Biosensum - Université de Montréal SensUs Team) to see what we will be up to. See you next September for SensUs 2019!

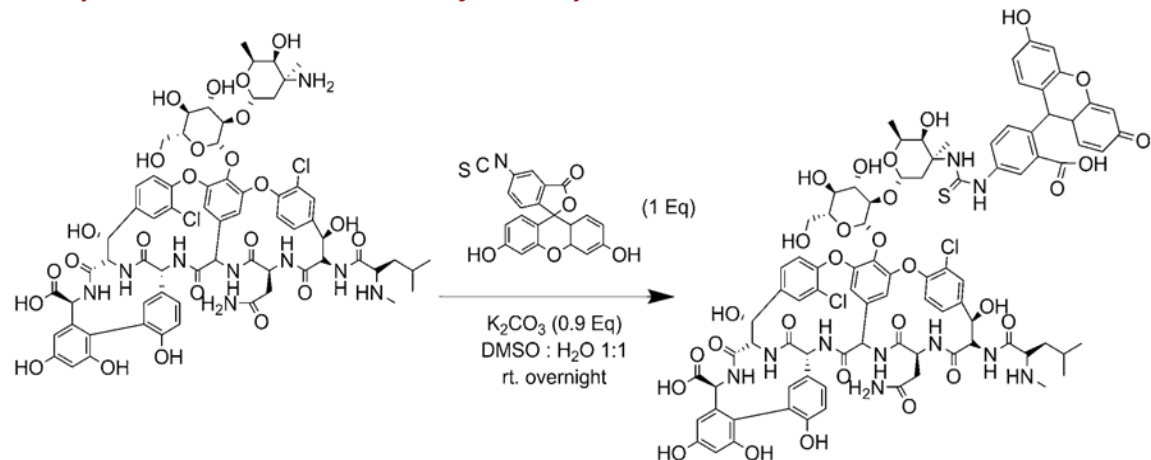
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## 9. Annexe

### 9.1. Synthesis and characterization of vancomycin-FITC

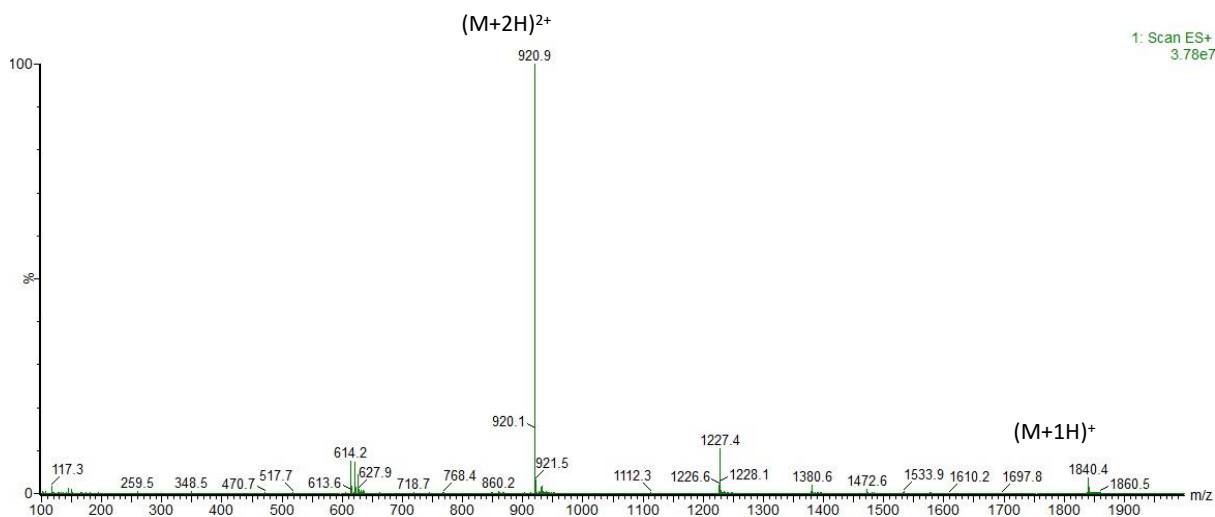


**Figure A1:** Synthesis of vancomycin isothiocyanate fluorescein

LC-MS prep method:

Column: XSelect@HSS PFP OBD 5  $\mu\text{m}$  19 mm x 100 mm

Elution: 24mL/min, 30:70 MeOH: $\text{H}_2\text{O}$ +Formic Acid 0.1% to 70:30 MeOH: $\text{H}_2\text{O}$ +Formic Acid 0.1% in 11 minutes. Collection of 920 m/z ( $\text{M}+2\text{H}$ )<sup>2+</sup>.



**Figure A2:** MS Spectra of Vancomycin-FITC

## 9.2. BiosensUM Complete Business Plan prepared by Antoine Nkaye

### Section One: Packaging of a testing service.

#### A. Description of the BiosensUM business orientations.

The BiosensUM mission is to provide added value to the health sector by creating an entire platform easily usable for a multitude of tests. Our short-term goal is the elaboration of a vancomycin tester, but in the long run we'll make our device as versatile as possible to efficiently answer the future market's needs. Currently, our main objectives are:

- Acquiring the ability to detect vancomycin concentration in less than 5 minutes.
- Creating a client-based approach to produce a user-friendly machine and the complementary resources around its usage.
- Elaborate differentiation techniques to retain customers, such as custom packaging and leasing testers.

The guiding strategy relies on a growth tactic based on adding new products into the market. There is always a need for technological improvement in the health sector, which makes it a priority for business board members. We want to show them how much added value (savings, productivity, etc.) they can get by adhering to our services. Our success will come from selling a faster tester that can be used by newly trained nurses. Indeed, technicians' work would therefore not be needed as much as right now. When it comes to the business strategy, it's mostly a differentiation approach since our platform is unique and we'll be adopting custom client services. Although, efficient cost managing is still a priority to keep us ahead of the competition. We'll discuss more deeply the current market, our marketing plan and other important points in later sections of the plan.

#### B. The tester and the resources surrounding it.

The packaging of our products can vary depending on the client's need. Our main product is the tester itself, but profit comes mostly from selling supplies related to the machine on a periodical basis. Also, it is possible to add expertise on the various types of technical implementation over the production process to add even more value to the business. Finally, we want to have a helpful guide on how to easily use our product, personalize pens, stickers and other furniture that draws attention to our brand. Our product needs to be seen as innovative and fun to use. Therefore, we are working on a design that draws attention and possibly adding a touch screen. At the beginning, we would rather be focusing on delivering a product that satisfies all our production criteria (laws, speed, validity of the test, resistance to water and usage, etc.) to then focus on a final model that would penetrate the market efficiently. Another important aspect of our service is the complementary equipment we can add to the bill. We will use some basic discrimination methods to drag most of the customer profit. One example of that is the concept of different bundles offered for different prices. We can also add some special orders (products we don't have in inventory) to the menu, which makes us able to earn money by selling goods from another merchandiser. Since our sensor can be used bedside by a nurse instead of having the test be performed in a laboratory by technicians, it gives faster results, which saves time, energy and money. Indeed, our customers will be able to implement a new service process that makes the business more efficient and helps them satisfy their

patients' needs. In the long run, our clients will also have access to a wide variety of chips for different tests, which will increase the versatility of the tester.

### C. Healthcare market analysis

Throughout the year, we have interviewed and surveyed dozens of stakeholders to deepen our understanding of the market. As a result, we now have a clear vision of the Canadian health sector's needs in terms of technological devices. The healthcare sector's need for technological devices is growing. Our approach must be adapted to two sectors (public and private) for which the strategy is quite different. Indeed, it is a priority for us to meet the requirements to obtain contracts in both fields.

Public sector: After interviewing biotech equipment decision makers, we obtained pertinent information about how to apply for contracts we want to obtain. In Canada, especially in Quebec, many requirements are necessary in order to obtain an agreement. Some of those include legal registration, full disclosure of financial statements and many more. Our objective is to obtain a no tender contract agreement, which lets us overextend the standard legal duration of a contract (3 years) to more. This is crucial for our supply-chain management strategy since it enables us to supply the client over the life of the patent.

Private sector: For the private sector, the more visibility we obtain, the better it is. This starts by an efficient marketing plan so that everyone knows about us.

### D. Marketing Plan

Application and Effect of Loan or Investment: Collecting the necessary funds to finance the research and development costs is important, a well-executed management of that money is mandatory. With the diversity of knowledge our team has and the help of a few creditors, we can draw attention to our brand, retain customers and work towards great earnings over time. We are confident in our ability to put our tester into the client's hands.

### Section Two: Financial Data.

Chips			
Item	# of units	Price per unit	Total cost
Microbead	1	0	0
EDC/NHS	1	0,1	0,1
Antibody	1	8	8
Vancomycin	1	0,33	0,33
Solvent	1	1	1
FITC	2	4	8
MS separator	1	16	16
Buffer	1	0,1	0,1
Total cost per chips			33,53

Microfluidic montage			
Item	# of units	Price per unit	Total cost
Tubing	1	2	2
Connectors	14	3	42
Valve	2	1500	3000
Syringe driver	1	0,2	0,2
Syringe	1	1	1
Total cost per chips			3045,2



Device			
Item	# of units	Price per unit	Total cost
LED	1	200	200
Collimator	1	270	270
Connecting joint	19	2	38
Lens tube	1	20	20
Dichroic mirror block	1	165	165
Converging lens	2	48	96
Filter	1	305	305
Dichroic mirror	1	255	255
Raspberry-Pi	1	45	45
Electric wire	20	0,057	1,14
Breadboard	1	10	10
Digital oscilloscope	1	250	250
Detector	1	1117	1117
LED screen	1	35	35
Pump pieces	2	45,5	91
Total cost per unit			2898,14