Team Results Document
SenseWURk

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Traumatic Brain Injury

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UNIVERSITY & RESEARCH

SenseWURk '23
WUR Sensing It!

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1. Abstract

Lateral flow immunoassays are currently one of the most effective and applicable methods for point-of-care testing. We have developed a biosensor based on this technology that can detect and quantify the severity of traumatic brain injury (TBI). In our test, we use an immunoassay labeled with europium nanoparticles, which can detect the biomarker Glial Fibrillary Acidic Protein (GFAP). The ensuing signal is measurable via fluorescence using a dedicated reader. According to theoretical calculations and experimental data, our test should be able to detect the given concentration ranges of the competition. We prioritized developing an affordable, accessible, and accurate test according to the needs of healthcare professionals. Furthermore, our test aims to enhance diagnostic sensitivity by objectively quantifying TBI severity and addressing the spectrum of mild-to-moderate cases, which are frequently overlooked by CT/MRI scans. With production costs as low as €0.55 per strip, only the test strip is disposed, making it a cost-effective solution. After finetuning and optimizing an immunoassay for lateral flow, the test is currently able to provide a semi-quantitative determination of GFAP concentrations in a sample of interest.

2. Biosensor system and assay

In this section, we will explain how the Glial Fibrillary Acidic Protein (GFAP), the analyte, is measured in our biosensor system. GFAP is a clinically relevant biomarker for TBI. Within the first hours of TBI, elevated levels of GFAP in blood can be detected, which peak at about twenty hours of injury, after which they gradually decrease. Its concentration correlates to the severity of TBI. Our biosensor is based on a lateral flow immunoassay, which is an established and widely used technique for biosensing (Koczula & Gallotta, 2016). This technology provides results in minutes rather than hours as compared to a conventional immunoassay format like ELISA.

2.1. Molecular recognition and assay reagents

In short, a thin detection line has been coated with a primary antibody that can capture the analyte and labelled secondary antibody that flow due to capillary forces. Visual or instrumental analysis of the line area then allows for the determination of the analyte concentration. In our test, secondary (detection) GFAP antibodies are fluorescently-labelled and present on a conjugate part of the lateral flow assay. When GFAP is present in the sample, the secondary GFAP antibodies will bind to GFAP and form an analyte-conjugate complex. Primary (capture) GFAP antibodies are coated on a detection line on a nitrocellulose strip to bind to our target analyte GFAP and/or the analyte-conjugate complex and accumulate on the detection line. This mechanism is shown in Figure 2.1. Excess non-bound antibodies/particles are transported away from the detection line through lateral flow. The analyte can then be detected and quantified by a fluorescence reader instrument (Section 2.4). To determine the concentration of the sample, a calibration curve is made in which the intensity of the emitted light will relate to the concentration of the europium particles and thus of the amount analyte present.

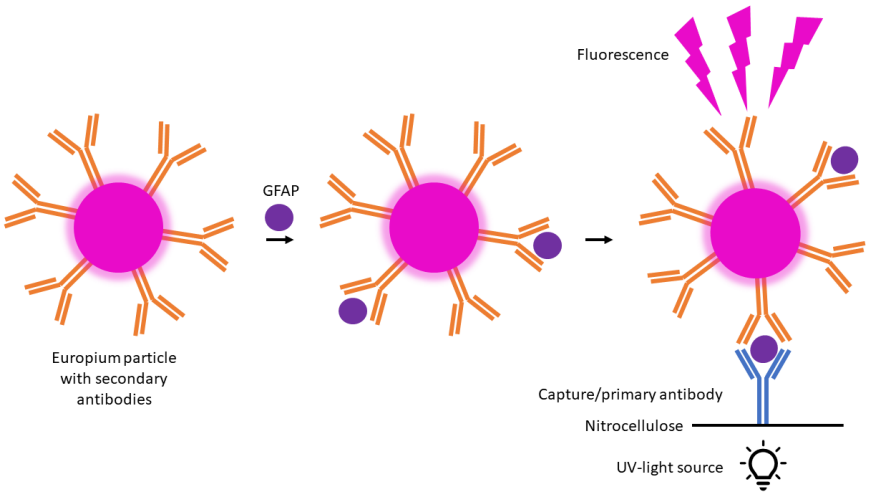


Figure 2.1: Schematic representation of the molecular recognition system. Europium nanoparticles are coated with secondary GFAP antibodies. The secondary GFAP antibody is coated on nitrocellulose. Upon binding of GFAP to the primary and secondary GFAP antibody and UV excitation, fluorescence can be detected and measured in the detection region.

The secondary antibodies are bound to Europium nanoparticles (EuNPs) of around 200 nm in diameter, which acts as our fluorescent marker. This antibody-europium conjugate is formed by carboxyl-to-amine crosslinking using the carbodiimide EDC and sulfo-NHS, as shown in Figure 2.2.

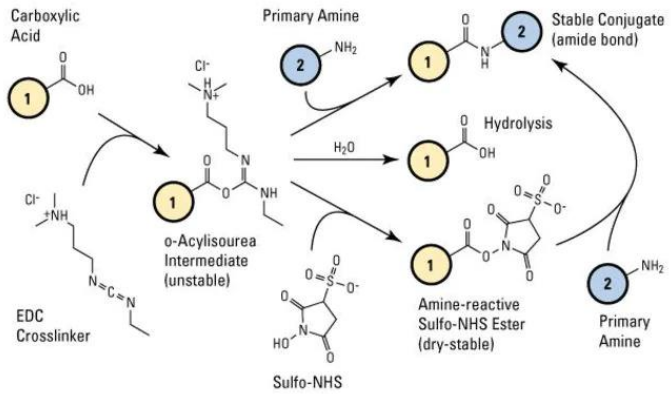


Figure 2.2: Carboxyl-to-amine crosslinking using carbodiimide EDC and sulfo-NHS. Molecule 1 represents the EuNP and molecule 2 represents the secondary antibody to be coupled. (VWR, n.d.)

2.2. Cartridge technology

The configuration of the test strip is shown in Figure 2.3 and is similar to other lateral flow assays. These strips are composed of several components: a sample pad for placement of the sample of interest, a conjugate pad that contains the EuNP-antibody conjugates, a nitrocellulose strip featuring a test line of primary GFAP antibodies, and an absorbance pad that ensures unidirectional flow by absorbing the fluid. Additionally, an alignment line is printed on the strip to ensure correct alignment of the strip into the cartridge and with the reader's sensors. Lateral flow immunoassays use capillary forces to transport fluid containing an analyte of interest and labelled particles through a porous strip of nitrocellulose (NC). The sample is diluted 5 times in running buffer to transport fluid containing an analyte of interest and label particles through a porous strip of nitrocellulose (NC). The cartridge is a 3D-printed plastic holder into which the assay strip is placed (Figure 2.4). This cartridge with strip can then fit into the reader instrument for measurement (Section 2.4). The plastic cartridge can be washed and re-used, the assay strip within is disposable (See section 5.3.4 for a consideration of sustainability).

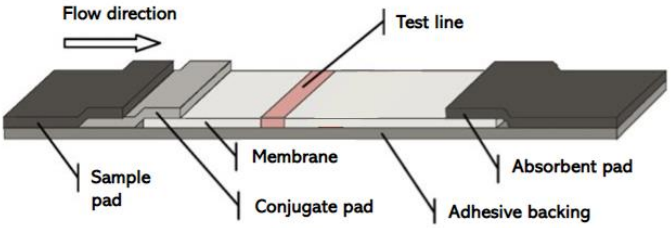


Figure 2.3: Typical configuration of a lateral flow immunoassay assay strip. From: Koczula & Gallotta (2016).

2.3. Physical transduction

When the analyte is present, the EuNPs will be present in the detection zone of the sensor. UV-Light will be used to excite the EuNPs at a wavelength of 394 nm, resulting in fluorescence (see Figure 2.2). The intensity of the emitted light will relate to the concentration of the europium particles and thus of the analyte. Since europium has a longer life-time of fluorescence, as compared to autofluorescence of the other materials, background can be filtered out when exciting the sample with an LED. This allows for time-gated measurements, where only the fluorescence of EuNP is detected, making the test more sensitive.

2.4. Reader instrument and user interaction

We utilize a proprietary fluorescent reader, provided by ams OSRAM, for our testing purposes (Figure 2.4). This reader is equipped with multiple sensors and photodiodes that can excite the sample using eight different wavelengths, while also having ten channels for measuring emission. It is specifically suited to measure the wavelength spectrum of Europium nanoparticles (394 nm), which serves as our method of detection. With the capability of conducting and processing up to 20 measurements per second, this reader is exceptionally fast and powerful.

In terms of physical attributes, the reader's measurements are 6x4x2 cm, and it weighs only 62.7 grams. As a result, it is compact and lightweight, making it ideal for point-of-care testing. The reader is enclosed by a 3D-printed outer case into which 3D-printed cartridges for the lateral flow strips fit. To obtain accurate signals, it is crucial to align the test strips properly with the reader's sensors. A colored line printed on the nitrocellulose strips serves as an alignment line. This ensures correct alignment of the test's detection line with one of the three the detection slots of the reader and cartridge.

Setting up the reader for the user is a straightforward process, as the necessary software is already provided and compatible with most PCs. The user simply needs to insert the cartridge, prepare the sample, add the sample onto the cartridge, initiate the measurement, and export the data. Furthermore, mobile app compatibility is currently being developed, which will further enhance user-friendliness. For the purpose of this competition, data analysis is conducted in Excel using a pre-established calibration curve.

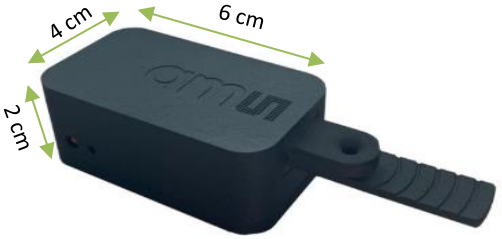


Figure 2.4: Image of the ams OSRAM fluorescence reader, into which a lateral flow strip cartridge has been inserted.

3. Technological feasibility

To show that our test is able to detect samples in the concentration ranges given by the competition, we have considered the theoretical background of lateral flow and supported our experiments with calculations. While lateral flow serves as a rapid point-of-care test, our focus on quantification and enhancing accuracy has led us to explore several key factors: the properties of nitrocellulose (NC), the particle dimensions of GFAP and conjugate, their respective concentrations, and the reader sensitivity. Additionally, we will present preliminary experimental data for our current biosensor design.

To enable to demonstrate low concentrations of antigens, the capture efficiency in the coated line must be high and the residence time for GFAP molecules and label particles long enough. The residence time is partly determined by the velocity of capillary flow, which can be described by:

$$v(t) = r\gamma \cos\theta/(4\mu L(t)),$$ where

v = velocity of capillary flow [m s^{-1}]
r = equivalent radius of a capillary tube (in our case the nitrocellulose pores) [m]
γ = fluid's surface tension [N m^{-1}]
θ = contact angle between solid NC and fluid, in this type of NC close to zero [°]
μ = fluid viscosity [N s m^2]
L = length of wetted NC [m]

From this formula we can derive that the further the fluid penetrates into the NC (increased L), the lower the velocity of the fluid. The average pore radius is a property of the NC, of which varieties are available with distinct smaller and larger pore dimensions. Other parameters in the equation are fluid properties of plasma, which are similar to those of water due to dilution in a buffer solution. The location of the absorbance pad relative to the nitrocellulose may also affect the velocity of capillary flow. However, due to fixed dimensions of the cartridge, fluid velocity cannot be altered this way.

Therefore, a key parameter under our control was the choice of nitrocellulose type. We chose a low speed class of NC, as it allows for a longer residence time for the molecules. With its small equivalent radius, molecules/particles in the fluid are always close to the NC's solid phase when passing the capture line.

Our assay is optimized to allow for maximal encounter probability between the analyte-conjugate complex and the coated line. This is dependent on the diffusion distances of both GFAP and Europium and the approximate distance between fluid and NC. Due to its porosity, NC materials have a total surface-to-fluid interface area in the order of 100 times the cover surface area. This results in an average fluid layer thickness in the micron range (see Figure 3.1). The thickness of this layer can be calculated using the properties of the NC and fluid.

Next, we can use the approximate diffusion distances of GFAP and Europium conjugate to roughly judge the probability of a particle hitting the NC and therefore binding to the coated line. For this, we used the Stokes-Einstein equation:

$$D = \frac{k_B T}{6\pi\eta r},$$ where

D = diffusion coefficient [m^2/s]
k_B = Boltzmann constant [m^2 kg s^{-2} K^{-1}]
T = absolute temperature [K]
η = dynamic viscosity [Pa s^{-1}]
r = radius of the molecule [m]

By estimating the material's residence time in the line area at around 2-4 s, the diffusion distance $\sqrt{D \cdot t}$ exceeds the layer thickness. This means that the probability of encountering the capture molecule-covered solid phase and being captured will be very high.

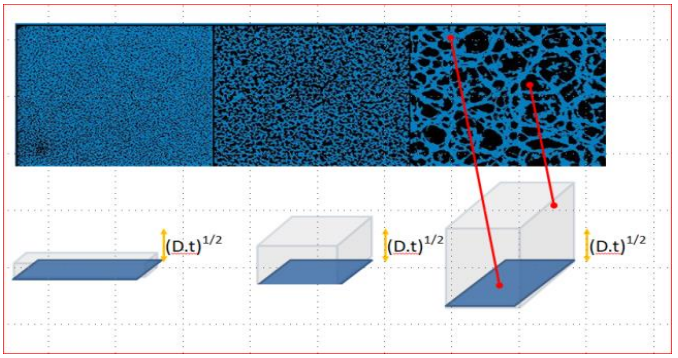


Figure 3.1: Illustration on how the fluid layer thickness is affected by NC pore characteristics (van Amerongen & Beumer, 2022)

To realize our detection ranges, we calculated the amount of coating, conjugate and analyte to support the feasibility of our test. Our GFAP detection range requirements are 100 pg/mL to 10 ng/mL, which results in **1.2 x 10^8** to **1.2 x 10^{11}** GFAP molecules per mL. Ideally, low binding chance between the antigen and the detection molecule will lead to more binding of the antigen on the coated line, which will lead to overall increased sensitivity (Gasperino et al., 2018). Therefore, the concentration of conjugate particles should be high enough to create a complex at the detection zone; however, excessively high concentrations of conjugate particles will result in reduced binding affinity.

In our test, we have calculated and tested an optimum particle coverage with secondary antibodies of **188** and **0.19** molecules of GFAP per antibody. This results in a maximum particle density in the detection zone proportional to the number of GFAP and at its best lying between **4.50 x 10^5** and **4.80 x 10^8** particles in our detection line. The reader's sensitivity range is given at **1.8 x 10^4** to **5.6 x 10^5** particles per line (see Figure 3.2). Assuming that not all particles are able to bind at 100% efficiency, there is considerable overlap between the detectable ranges of our test. Therefore, the reader should be sensitive enough to detect the samples in the given concentration ranges.

Particles per test line [total counts]	LFT Test Strips with different sample concentration level printed to the LFT strip
5.6×10^5	
2.8×10^5	
1.4×10^5	
7×10^4	
3.5×10^4	
1.8×10^4	

Figure 3.2: Table showing the ranges of EuNP particles per test line. The purple line corresponds with the given signal intensity at a certain particle count (for illustration purposes only, fluorescence is not visible as shown here) (Derived from a demo presentation of ams OSRAM).

In short, we have chosen our NC and estimated coating according to the requirements of this competition. According to theoretical calculations, our assay and reader should operate well within the given ranges of GFAP concentrations in conventional TBI patients. Our experimental results have shown that we have a clear and strong signal with different GFAP concentrations up until 1.25 ng/ul at the time of writing (Figure 3.3)

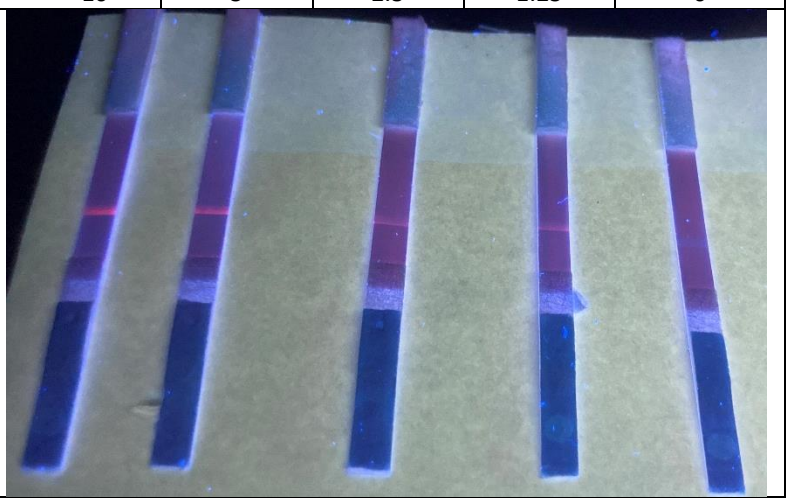
GFAP (ng/µL)	10	5	2.5	1.25	0
					

Figure 3.3: Preliminary experimental results of lateral flow strips with GFAP concentrations 10, 5, 2.5, 1.25 and 0 ng/ml. The intensity of the pink line (europium bound to detection line) correlates with decreasing GFAP concentrations, while no background signal is found at 0 GFAP ng/ml.

4. Originality

Here the novelties of the biosensor are described together with the contribution by the team and by people outside the team. Both the team captains and the supervisor have written a piece and signed this page to confirm that all statements provided are true.

4.1. By the team captains

Lateral flow is currently a widely used effective and applicable method for point of care testing when screening for injuries or diseases. Our developed biosensor expands upon this technology. For example, in comparison with the lateral flow assays on the market, our sensor is quantitative due to the use of a fluorescence reader instead of a visual signal like the Covid-19 lateral flow test. Fluorescence is also a novelty making the sensor more sensitive than other currently available colorimetric systems since the background intensity can be filtered out. This fluorescence arises from europium nanoparticles (EuNPs). To process the data of the reader, we developed an Excel template to transfer this data into the concentration of GFAP and thus the severity of TBI. This template makes use of the increase in fluorescence over time, which correlates to the EuNP concentration, during the measurement, causing the measured concentration to be even more accurate. Furthermore, we optimized our lateral flow assay by varying different parameters. First of all, we found that the best antibody pair in our sensing system was not the one that was recommended. Next to that, we investigated the use of different nitrocellulose membranes, buffer compositions and concentrations of capture and detection antibodies. During the development of our test, we have received expert help and advice from the following individuals: Tom Beumer, a physicist in the field of protein kinetics, who helped us with estimating which concentrations of antibodies and other molecules would theoretically be best for our assay to measure the entire GFAP concentration range that is set by the competition; Heleen van den Bosch, a researcher at Wageningen Food & Biobased Research, who developed the protocol to couple EuNPs to antibodies; and our partners and coaches.

4.2. By the team's supervisor

After initially trying to develop a microfluidic chip assay, the students finally settled on refining the well-known concept of lateral flow immunoassays for detecting GFAP (Glial Fibrillary Acidic Protein) as a marker for TBI (Traumatic Brain Injury). In contrast to non-quantitative assays such as the standard Covid test or common pregnancy tests, the assay developed here uses fluorescent Europium nanoparticles conjugated to secondary antibodies allowing the efficient quantification of GFAP levels in human blood. As a device for read-out the fluorescent signal, a small handheld device (provided by ams OSRAM) is sufficient, enabling cost effective and sensitive detection.

The students tested multiple pairs of primary antibodies and secondary antibodies labelled with fluorescent Europium nanoparticles. For the design of the flow assay, different nitrocellulose membranes were tested, as well as different ways of sample pretreatment. The students were able to showcase a clear relation between sample concentration and signal intensity, supported with theoretical calculations. The students further developed an Excel Work Sheet that allows reading the data from the handheld device. Combined with a calibration curve, an appropriate concentration of GFAP can be estimated and categorized into levels of TBI severity.

Basic knowledge on developing lateral flow assays was provided by "BioSensing & Diagnostics" at Wageningen Food & Biobased Research. Experiments and development was performed at the "Laboratory of Bionanotechnology" at Wageningen University & Research with support from lab members. Frequent meeting involving the team members and coaches/supervisors took place in offices provided by the WUR student challenges. Financial support was provided by WUR Student Challenges, AFSG WUR, and the Hersenstichting. Kenosha provided tape samples. Jobst Technologies provided a pump, pump drivers and tubing for initial testing using a fluids based assay. Ams OSRAM provided the reader and training. Cytiva provided nitrocellulose membranes and the Wageningen Fablab helped with a prototyping workshop.

Sifre van Teeffelen

Sophie van den Boom

Johannes Hohlbein

5. Translational potential

5.1. Introduction and proposed business model

Traumatic Brain Injury (TBI) is an injury to the brain caused by sudden external forces such as a falls, assault, explosions, or vehicle-related accidents. TBI affects an estimated 70 million people worldwide annually. The injury can be classified as mild, moderate, or severe, with most cases (~81%) being mild. Depending on the severity of TBI, these TBI patients suffer symptoms including memory loss, cognitive and speech problems, headaches, and fatigue, which can last up to a lifetime. A fast and accurate diagnosis of the severity of TBI and appropriate treatment following injury is crucial for their recovery (*National Institute of Neurological Disorders and Stroke, 2023*).

Unfortunately, diagnosis is a clear bottleneck in the TBI patient journey (Appendix I.I). Currently, TBI is diagnosed using the Glasgow Coma Scale (GCS) and cognitive tests, which aim to describe the extent of impairment suffered by the trauma patient, as well as by neuroimaging by MRI/CT scans (Appendix II). The main bottlenecks of these methods are that the procedures can be time-consuming, labor-intensive, imprecise, and do not detect all cases of TBI, especially the mild to moderate ones (*New York State Department of Health, 2018*). As a result of a prolonged time between injury and treatment or a undetected and/or inaccurate diagnosis, the time and final status of recovery can be negatively affected. In turn, this is disadvantageous for all stakeholders involved (Section 5.2).

As a result, tools are needed for the rapid and more accurate diagnosis of the severity of TBI, so that appropriate treatment can be given, and the lives of TBI patients improved. We propose to introduce a lateral flow point-of-care biosensor as an additional diagnostic method alongside current methods. To be able to introduce the biosensor to the market, we developed a business model, as shown in Figure 6.1. The problem and solution statement are already quickly covered in this introduction. Explanations that elaborate on the business model are further covered in this chapter and divided into the following subsections: stakeholder desirability, business feasibility, and financial feasibility.

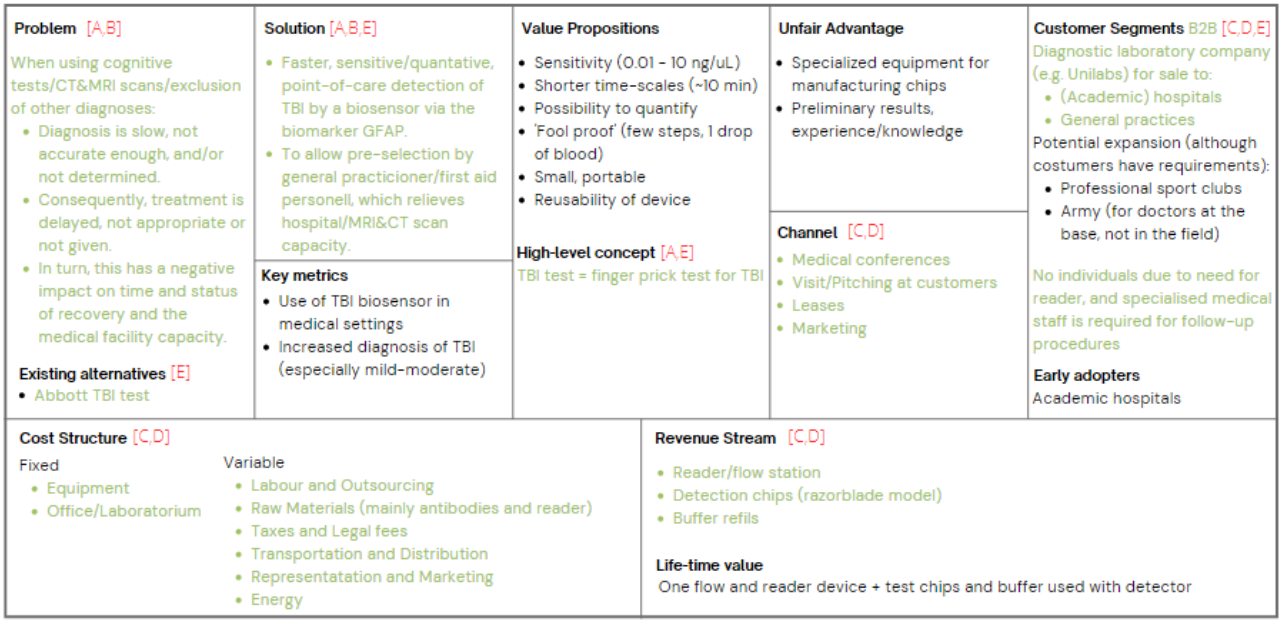


Figure 6.1: Business model canvas for the GFAP lateral-flow biosensor for TBI with validations by potential key stakeholders (A-E) indicated in green as also indicated under support (Section 5.4).

5.2. Stakeholder desirability

5.2.1. Stakeholders and costumers

Various stakeholders can be identified within the TBI patient journey (Appendix I.I), who all have different needs and relations to the product. Firstly, we identify the patient itself, who ideally wants to be diagnosed and treated by health care personnel in a quick, accurate, cheap, and non-invasive manner. This would ideally result in a comfortable hospital experience and complete recovery. In case of severe symptoms, the patient would benefit from proof of their inability to work due to TBI in the form of a (more objective) diagnosis. Indirectly also their health insurer, employer, the tax-payers, and the government can be identified as stakeholders, as they benefit from a clear process with short duration and/or full recovery for the patient to save (long-term) costs.

Secondly, various stakeholders within the medical field can be identified. This includes the hospital, the hospital's diagnostic laboratories, and general practitioner offices, as well as their employed medical professionals involved in the TBI patient journey. Regarding the health institutions, they desire to ensure a pleasant and suitable treatment for the patient, like their personnel and the patient. However, they need to consider the hospital's capacity, and efficiency and costs of methods. This means that the biosensor must contribute to cheaper and more efficient diagnosis to correspond to the needs of the health insurer and patient.

Regarding the health care personnel, their tasks within the patient journey are very diverse and can be divided into subsections. General practitioners and ambulance personnel generally are the first to respond and have limited access to technology to accurately diagnose a patient. They could benefit from an easy-to-use, small, quantitative method to support an initial diagnosis. This would allow them to refer the patient to the accurate specialists. ER doctors could benefit from the biosensor to give rapid results with minimal and straight forward handling during an emergency to decide what and whether further care is needed. If further steps are taken, a neurologist could give a more supported diagnosis based on the results of the biosensor, neurological exam, and the results of MRI/CT scans together. In addition, this test will provide more sensitive information about the severity of the TBI, which is an improvement in comparison to current methods, since CT or MRI scans are often not able to detect mild and, sometimes, moderate TBI. An additional benefit is that the strain on the hospital's capacity would be relieved if TBI could immediately be ruled out, for instance by decreasing the amount of (unnecessary) CT/MRI scans made. Thus improved diagnosis by the personell would positively affect the hospital itself.

Of all the stakeholders, we identified three different customers. We identified that the customer of our biosensor will not be the patient but (the diagnostic laboratory of) the hospital or the general practitioners' office. This makes our business model business-to-business. In addition, employers of risk groups for TBI such as the army and sports clubs have been identified.

For all customers the bottleneck is in the diagnostic process. However, the way this bottleneck manifests can differ. Consequently, the desired solution(s) differ for the various customers. The hospital mostly desires a quick, less-labor intensive, sensitive and cheap biosensor of diagnosing suspected TBI patients that get admitted to the ER compared to current neuroimaging or diagnostic methods to improve the use of the hospital's capacity. Basically, a way to quicker triage a patient on TBI. General practitioners require an easy-to-handle, more accurate method than the currently used anamnesis and Glasgow Coma Scale to better determine if the patient needs a referral to a hospital, which can reduce overtreatment or unnecessary referral 'just to be sure'. For the army or a sports club, a field doctor needs to quickly decide if the patient should be removed from the (battle)field or can carry on. Thus, the biosensor should be easy-to-use, small, quick and give an indication of TBI.

Hospitals will be our main target group to market our biosensor in terms of potential, market volume and economic viability. Profitable customers are defined as a group that currently experience a gap or incomplete way of diagnosing patients with suspected TBI. This customer needs to have a general understanding of medical devices, how to operate them and how to interpret the results to give an accurate diagnosis. Supported by our validations, this matches the profile of medical institutions (Section 5.4). Making our test available for at-home use does not match in our view, also because TBI is too serious of an injury to develop at-home diagnostics for. This is further supported by the market opportunity navigator (Appendix I.II). Employers of risk groups are identified as a side market due to the higher risks involved, in addition more problem-solution validation is required.

On the other hand, various validations, certifications, and regulations are involved in the medical market. These regulations protect patients and ensure reliability of the biosensors. To gain these certifications, rigorous testing and clinical evaluation is needed. Regulations depend on the geographical area in which they are sold (McGrath & Scanaill, 2013). Our target market would be starting in the Netherlands, followed by Europe and North America, therefore we focus on european CE marking. When a product has a CE marking, it complies with EU legislation and can be sold within the European Economic Area (EEA). For a biosensor, directive 98/79/EC (*McGrath & Scanaill, 2013*) needs to be followed. Also, providing user information on performance & limitations is mandatory. However, there are no standard evaluation protocols yet and there is no transparent reporting of clinical data.

To attract investors and reassure the customers and stakeholders, additional qualifications, certificates and/or a patent are desired. For example, a quality management system (QMS) is an approach to process product quality management. This consists of International Organization for Standardization (ISO) standards. ISO 13845 is the most common for medical devices (*McGrath & Scanaill, 2013*). Furthermore, the stakeholders should be assured that their data is securely stored.

5.2.2. Value proposition

The current biosensor prototype can meet most important value properties, including being fast, small, quantativ, and sensitive (Chapter 2,3). However, optimization is needed for processing data and obtaining the results could be faster. As described earlier, the data is now processed using an excel sheet, however this will be more user-friendly if a proper program is written. Additionally, we would like to develop our own reader, however the current reader is suitable and appropriate.

To date, only the I-STAT TBI plasma test from Abbott is already approved for clinical measurment of TBI using GFAP as a biomarker (*Abbott, 2021*). Next to GFAP, the biomarker ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) is used in the I-STAT device, multiplexing is an improvement in comparison to our device. Their device uses a blood sample that is placed in a test cartridge and will give results in 15 minutes. The chip is sold for $16. This test is, however, not yet fully implemented into the health care system and mainly focusses on the U.S, compared to the EU for our case. The sensor is comparable in size, usability, speed, and sample size. The detection range of the i-STAT is 30 - 10000 pg/mL GFAP. Thus, the I-STAT test for TBI is only for mild TBI, which is less sensitive than the sensor presented here. Also, according to the website of Abbott, their sensor is not intended for point-of-care usage (Abbott, 2023). The precise detection mechanism of this biosensor could not be found and can't thus not be compared to our proposed biosensor. In our conclusion, this is strong competition, but it is still profitable to proceed with bringing a new biosensor for TBI on the European market.

5.3. Business feasibility

From here onwards, we consider a scenario where, following the SensUs competition, we continue as a start-up that aims to get their biosensor used by a couple of Dutch hospitals and general practitioners, followed by growth towards the Dutch market and eventually the European international market. Our main focus remains on hospitals and general practitioners but might be expanded to side identified markets such as sports clubs (Section 5.2.1).

5.3.1. Key resources

Various resources are required for development and production of our biosensor. First, our business model would rely on human resources. Following the competition, we would need to further develop and optimize our biosensor. To achieve this, we would need experienced knowledgeable scientists (partly us and already acquired network). In addition, ideally, we would develop our own reader, for this we would need technicians. Development would ideally be done in co-operation with the user, as this would also decrease the time to market. Moreover, we would need a sales and legal force, we would need to become more knowledgeable about these aspects, or this could be outsourced. The human force would need a physical office and laboratory. In addition, the office would need hardware and the laboratory would need the equipment necessary to produce and analyze our test. Hardware would include PCs to process the obtained data and optimize the read-out program. Lab equipment can vary from specialized machines to pipets. During our first stages, it is possible to pay the university for the use of their equipment. Later on, this would need to be rented or bought. We expect that at first, the biosensors could be produced in-house. But following up-scaling, we might need a manufacturing facility and distribution lines. To enable the previously mentioned resources, we would need financial resources. We would aim to obtain these from biotechnology start-up funding and investments. To improve our chances of funding, but also for legal reasons, an increasingly important part of our attention would be focused on obtaining certifications for our sensor for human clinical use and a patent. However, a patent might be difficult to obtain and is very resource intensive.

5.3.2. Key activities

Our key activities include actions for us to successfully operate and grow. First, our focus would be on optimization our of product and obtaining validations and certificates to reach the market as soon as possible. After market clearance, the first entry point of the biosensor is to bring it to the attention of experts in the field of TBI. This is done in ways of email, attending conferences and by approaching authors of medical trade journals such as the Dutch 'Nederlands Tijdschrift voor Geneeskunde'. During our interviews, dr. B. Jacobs already gave a sign of interest and sees great potential in using a biosensor such as our proposed one. By keeping him close in our network, we see an entry point to get in contact with more TBI neuro-specialists. Market entry to general practitioners and laboratories is done in the same way. Bringing the existence of our biosensor to the attention of the customers is in all cases the first step. By introducing potential customers to our biosensor with a trial, the first step of adaptation will be made easier. Another key activity is looking into obtaining a patent as this has currently not been done extensively. Obtaining a patent is a time consuming and expensive process. After filing for a patent, the information becomes public, although, no one else has the right to recreate. Another option is to be extremely secretive about the findings and workings of the biosensor. Although there is no applied patent on the technology, the possibility of recreation by a competitor will this way be held to a minimum.

5.3.3. Key partners

Key partners according to our business model are the suppliers of all the components of our lateral flow strips. Furthermore, the supplier of our currently used read-out device AMS osram is also considered a key partner in the current state of our sensor. In further optimalizations we strive to create our own optimized version of this reader. As of right now, the components are only bought in small quantities, making us only a small customer for our key partners. In later stages, these components will be bought in bulk, making us an important player for the suppliers. This creates a situation in which both parties have a win-win in terms of stable supply and demand contracts. Also, StartHub Wageningen is an institute that helps students, PhD's and recent graduates of Wageningen University & Research with their startups. When receiving help, they can provide valuable connections and give knowledge on how to proceed.

5.3.4. Sustainability

Our lateral flow has disposable components, namely the test strip and cartridge. This might seem wasteful, but overall, an lateral flow strip is quite sustainable to manufacture, as the strips are paper-based. This is also one of the reasons why they are so popular (Sena-Torralba et al., 2022). On the other hand, the Sustainable Healthcare Coalition has calculated that one test has about 5% of the carbon footprint of a GP visit (Morris & Haworth, 2022), which is considered high. Of this carbon footprint the highest contribution is by the cartridge and the extra packaging. As such, there must be solutions for this. One thing that can be done is the usage of biodegradable materials, such as PBAT or PLA, which are already used by SureScreen Diagnostic (Metcalf, 2022). A different strategy could be to provide our customers with proper instruction and recycling bins in which they can dispose of their cartridges. Additionally, pre-filling the running buffer vials, putting more tests in one lot, and not individually packaging every test in plastic will make the business more sustainable (Morris & Haworth, 2022). From a production point of view, our biosensor doesn't need many materials. For the materials that we use, it is best to buy them in bulk, and produce in bulk as well to lower the environmental impact. If possible, the materials can be bought from more companies within the same country. By charging the costumers a fair, but profitable price for the products we deliver, we ensure that the business can continue to grow and develop into a durable company.

5.4. Financial feasibility

5.4.1. Costs projection

The total overview of the cost of a (start-up) company can be divided into employees and outsourcing, research and development (R&D), production and distribution, marketing and representation, and legal fees and taxes. Furthermore, there are fixed costs for equipment and laboratory and office rental, as well as variable costs for energy and raw materials of our sensing device. The development costs of lateral flow assays are estimated between $30,000 and $100,000 in case the target analyte and antibodies are available, which they are in our case (*Lateral Flow Immunoassay*, n.d.). While the total funding required by previous biosensing start-ups, assumed to cover all aforementioned costs, varied between €1-2 million (crunchbase, n.d.).

Table 5.1: Materials used in a testing strip, with corresponding prices per strip.

Material	Costs
Nitrocellulose	€0.30
Transparent backing	€0.18
Absorbent pad	€0.011
Sample pad	€0.011
Conjugate pad	€0.02
Conjugate antibodies	€0.004
Europium nanoparticles	€0.005
Capture antibodies	€0.01
Buffer	negligible
Total price per strip	€0.55

To specify the costs of our biosensor; our biosensor consists of a reader and single-use strips. These test strips are placed into reusable cartridges which fit into the reader device. In addition, buffer is pipetted together with the blood sample on the strip. Therefore, besides the strip itself buffer is needed to execute a test. The costs of the AMS Osram reader, which we obtain commercially, are estimated at ~€400, based on the market price of the AllTest Lateral Flow Test Reader from Scientific Laboratory Supplies of £499.00 (*AllTest Lateral Flow Test Read | LFT1000 | SLS*, n.d.). An overview of all materials and their estimated costs for the test strips are given in Table 5.1 and total to an estimated €0.55 per strip. A detailed description of how these costs are calculated can be found in Appendix III.

5.4.2. Sales price

To cover all costs made by a (start-up) company (Section 5.4.1), we propose to sell a testing strip for €10.00 and the reader for €799. This would yield a profit of €9.45 euro per strip and €399 per reader device, this matches the razor-blade model (section 5.4.4). The price of €10.00 per strip is reasonable, despite the low manufacturing costs, in comparison to other lateral flow assay strips on the market. For example, mass-produced Covid-19 test strips are sold for €3.00-€8.00 in the Netherlands, and pregnancy tests for €2.00-€10.00. Our proposed price is also able to compete against the i-STAT device from Abbott who sell their detection cartridges for about $16 each and their device for approximately $10.000 (Middleton, 2022). Aside from the strips, if we sell our reader device to all emergency rooms in the Netherlands, the profit

for the reader would be €23.920, as there are 80 emergency rooms in the Netherlands (*Acute Zorg, n.d.)*. This profit can be expanded with maximum €1.697.423 by selling the reader device to general practices, of which there are 5677 in the Netherlands, and even more by expanding internationally or to side markets.

5.4.3. Market analysis

Our market is estimated to be the biggest for medical facilities, including first hospitals and secondly general practitioners starting in the Netherlands. Hersenstichting indicates an approximate 47,000 TBI patients reporting to the ER in 2016 in the Netherlands (*Wat Is Traumatisch Hersenletsel? - Hersenstichting, n.d.*), of which 38,000 are mild TBI cases and 9,000 are severe TBI cases. Considering there are 80 emergency rooms in the Netherlands, 104 mild TBI cases are diagnosed at the ER every day. Internationally, there are 64-74 million cases of TBI estimated annually (*Dewan et al., 2018*). Of these cases, most are reported in North-America and Europe, although the burden may be larger in other continents, but not reported. Moreover, tests will be performed on patients, who will eventually not be diagnosed with TBI. Our biosensor would make an impact by decreasing the costs of diagnosing TBI by reducing the number of CT and MRI scans needed and the time to treatment, thus reducing hospital stay. Ideally, the indirect costs of TBI are also reduced, making it desirable to implement the TBI biosensor. Currently, the global costs of TBI are ~76.5 billion, of which ~11.5 billion are medical costs and ~65.0 billion indirect costs. In Europe the costs per patient are approximately: €77-268.85 for GP's, €538 for emergency ambulance, €460.40/day for hospitals or €1.751.50/day for IC's, €469/day for rehabilitation and, €1400 for monitoring (Scholten et al., 2014). Due to the Covid-19 pandemic, there is an increase in the point-of-care market, which had a total market size of 23 billion in 2021. Furthermore, at the moment there is only one competing TBI biosensor on the market, produced by the company Abbott (Section 5.2.2) *(Krausz et al., 2021*). Challenges of this market include the high standards for certifications and validations, while meeting the time to market/revenue before investments run out.

5.4.4. Revenue streams and business strategy

To create revenue, we propose to make use of the razor blade model. The reader is sold for an alluring price and one time use strips are sold separately. Next to that, revenue will be created by selling running buffer refills, and cartridges. However, at first, we propose a lean system, so that the biosensor can be improved in cooperation with the customer. This reduces the customer's risk and our time-to-market. A revenue prediction is estimated based on assuming that we sell our device to 1/3rd of the ERs in the Netherlands, and that our device is used to diagnose all mild TBI cases. The profit of selling a reader device to the ER's would be 26* €399 = €10374. The profit for testing strips is 35* €9.45 = €330,75 every day. The break-even point for an investment of one million euros would be ten and a half years. This investment money is based on the investment money of Scope Biosciences, a spin off from Wageningen University (Crunchbase, n.d.). The revenue can be increased and break-even point shortened by expanding our sales to general practices in the Netherlands, or to Europe, America and/or to other costumer segments (army/sports clubs). Alternatively, we propose a business vision. First, we would like to improve our biosensor by multiplexing and developing a reader. However, we could aim to detect multiple diseases and their biomarkers (max 3) that are essential to test first for on a trauma patient using one single chip.

5.5. Support

To validate our assumptions a total of nine interviews were conducted with experts in the medical and biosensing fields. Regarding the need, required properties, and implementation of the biosensor in an active setting (Section 5.1 and 5.2), interviews have been conducted with patients, medical professionals and TBI experts. The interviews led to the validation of the shortcomings in current ways of diagnosing TBI patients and confirmed the need for a biosensor to solve these deficiencies. Furthermore, the interviews gave insight into the biosensor customers and the correct position of implementation of the biosensor. In addition, help from SensUs partners Micronit and Unilabs were essential in understanding the properties of a sensor to create value. These interviews combined made it apparent how our proposed biosensor could make a difference in diagnosing patients with suspected TBI compared to currently used methods. For the business model, Carla van Heck, an employee of the department of Corporate Value Creation from Wageningen University and Research, helped us to understand what was needed to start a start-up. Furthermore, Scope Biosciences gave us a walkthrough of the process they first went through before becoming profitable. These interviews have helped us to outline our own business plan as described above. Summaries of the interviews are added in Appendix II. In addition, we had support from our partners and coaches. Moreover, various literature sources were used.

6. Team and support

6.1. Contributions of the team members

- Marrit Bosch: secretary of the team, part of lab team, and captain of the reader team.
- Sophie van den Boom: vice-team captain, part of the business team, and captain of the lab team.
- Mira van Leiden: part of the lab team, and part of the reader team.
- Kayleen Ma: captain of the innovation team, part of the lab team, and part of the reader team.
- Tessa Neef: treasurer of the team, captain of the business team, part of the lab team, and part of the PR team.
- Janne van Overbeek: part of the lab team and part of the innovation team.
- Gertjan Spakman: part of the PR team, part of the business team, and complemented the lab team during the summer.
- Sifre van Teeffelen: captain of the team, part of the lab team, and part of the business team.
- Bodhi Voss: captain of the PR team, and part of the lab team.

6.2. People who have given support

- Ruben Massop: helped us in the laboratory.
- Heleen van den Bosch: helped us in the laboratory.
- Tom Beumer: helped us with the technological feasibility calculations and supported us with the theoretical background.
- Kimm Borst: shared the knowledge of her Master Thesis and her participation in last year's SensUs project.
- Lutger Rutten: helped with the configuration of the reader and shared experiences of his participation in last year's SensUs project.
- Anton Bunschoten: helped us with ordering laboratory materials.

6.3. Sponsors and partners

- WUR Student Challenges: provided us financial support, offered us working space and multiple workshops.
- AFSG WUR: provided us financial support.
- Hersenstichting: provided us financial support.
- Hytest: provided us with antibodies and antigen
- Kenosha tapes: provided us laboratory tape samples.
- Jobst Technologies: provided us with a microfluidic pump, pump drivers, and tubing.
- ams OSRAM: provided us with the reader and a training on how to use the reader.
- Cytiva: provided us different types of nitrocellulose membranes.
- Ahlstrom: provided us different types of cytosep membranes.
- Fablab: offered us a prototyping workshop.
- The following people and organizations helped us by providing information through interviews:
    - Djan Mattijssen (TBI-patient)
    - Ingrid Hummelen (General Practitioner)
    - Nancy Heijne (General Practitioner)
    - Issam Boukrab (Neuroradiologist)
    - Bram Jacobs (TBI-specialist)
    - Arjan Tibbe (OnePlanet)
    - Carla van Heck (Corporate Value Creation of Wageningen University)
    - Jurre Steens (Scope Biosciences)
    - Angela Bikker (Unilabs)
    - Laura Folkertsma-Hendriks (Micronit)

7. Final Remarks

This competition was a very educative journey for all of the team members, and we have learned and grown a lot individually and as a team. We faced various challenges, for instance in the lab our initial idea for a biosensor had to be altered as it did not work as we initially expected it to. This really taught how research works and that you have to be flexible. This journey would not have been possible without the support of our coaches, the people who helped us in the lab, our partners and those people who wanted to be interviewed by us to answer questions and share knowledge. We would like to wholeheartedly thank you for your support. For now, we have no plans in developing our biosensor further, but we will definitely use our obtained knowledge in our future careers!

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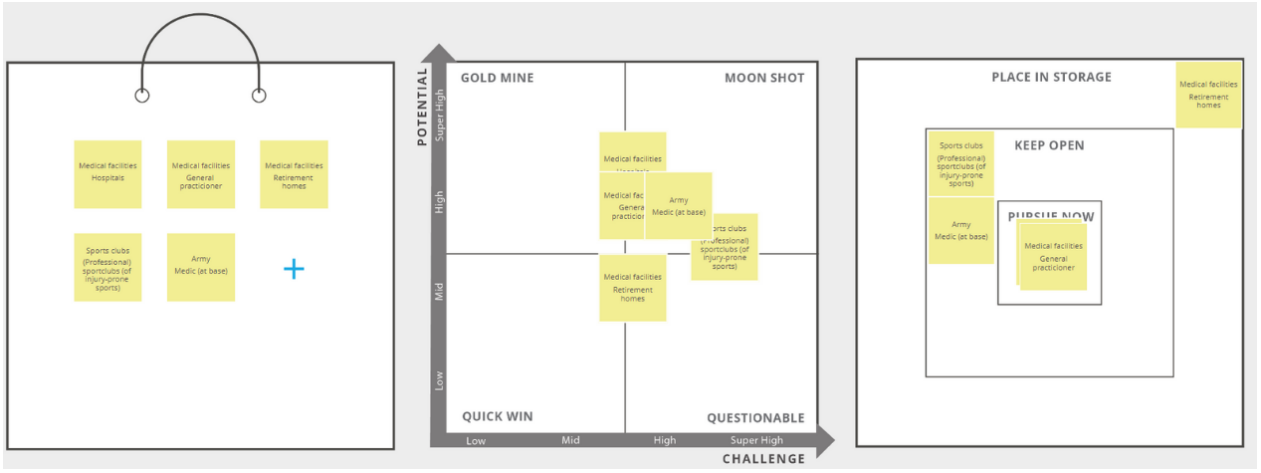
9. Appendices

I. Supplemental canvases translational potential

I.I TBI patient journey

	Pre-hospital visit	Visit hospital	Diagnosis	Treatment/recovery	Monitoring
Patients actions	Depending on severity: • Wait for an ambulance • Travel to general practitioner	Depending on severity: • Be in the hospital • Be at the general practitioner • Go to appointments	• Undergo tests • Wait for results/diagnosis	• Undergo treatment • Follow doctor's advice	• Go to appointments • Undergo monitoring • Follow doctor's advice
Patients experience	Depending on severity: • Admitted in ambulance • Travel to emergency room • Travel to general practitioner's office	Depending on severity: • Emergency room check-in • Appointment with neurologist • Appointment with radiologist • Appointment with general practicioner	Neurological exam: • Glasgow Coma Scale (GCS) • Loss of memory assessment • Loss of conciousness assessment Neuroimaging: • CT Scan • MRI scan	For mild-moderate TBI: • Rest • Avoid stimuli (e.g. sound, light) • Pain killers Additionally for moderate-severe TBI: • Surgery • Rehabilitation therapy • Further medication	Depending on severity: • Appointments with neurologist • Follow-up neurological exams, neuroimaging and/or intracranial pressure monitoring
Health personnel involved	Depending on severity: • Ambulance personnel • General practitioner and staff	Depending on severity: • Emergency room staff and hospital personnel • General practitioner	Depending on severity: • Neurologist • Radiology technician • General practitioner	Depending on severity: • Surgeon • Neurologist • Therapist (e.g. speech, physical, respiratory)	Depending on severity: • Neurologist • Radiology technician

I.III Market potential navigator



II. Summaries of interviews with various stakeholders

II.I Neuroradiologist (February 7th 2023)

We interviewed a neuroradiologist at Elisabeth TweeSteden hospital in Tilburg. He is specialized in assessing neurological CT/MRI scans and is therefore very knowledgeable of the diagnosis of TBI, especially regarding large traumas. When trauma patients are admitted to the hospital and it is obvious that they have some sort of brain trauma, they will first receive a CT scan. CT scans are a quick way to easily assess whether there are any skull fractures, internal bleeding, brain tissue damage and/or contusions. This will be compared to the current state of the patient. When a CT scan is not sufficient in explaining the patient's behavior (for example no clear indication of when the patient is waking up from a coma), an MRI will be used. MRI scans are much more sensitive than CT scans and can pick up small brain injuries. These detection methods are mainly used in severe TBI cases, since they are costly, time consuming and require specialists. MRI scans are only performed if necessary, as an MRI requires a patient to lie still for a long period of time and is very costly. For mild cases of TBI, brain imaging is not used, and mild concussion will often be diagnosed at the ER. Misdiagnosis is extremely rare, as the severity of TBI cases is usually easy to determine from the patient's experiences.

II.II TBI patient (March 15th 2023)

An interview was conducted with Djan Mattijssen, a 25 years old TBI patient. She had a bicycle accident in 2016 and was diagnosed with TBI. She was brought to the ER by an ambulance after her accident. An MRI was taken, and she had to spend the night in the hospital. The weeks after the accident she had a lot of headaches and was very sensitive to sound and light. She also had trouble sleeping. The diagnosis presumably TBI was give the night/morning of the accident by a neurologist based on the MRI scan and cognitive research. The recovery advice the neurologist gave her was no/very little stimuli for around two months. Now, years later, she still has more headaches than before the accident and she is still sensitive to sound. She sees added value in our sensor. Especially because the MRI scan did not give a clear diagnosis for her, and her diagnosis was partly based on a questionnaire. Here every person would rate their pain differently, this is very subjective. Also, she thinks it can be very useful to know the severity of the TBI, also to be able to give better recovery advice. With this interview we gained more information about the diagnosis steps of TBI and the experience of the patient.

II.III TBI specialist (April 11th 2023)

Bram Jacobs gave great insights on how TBI is currently diagnosed and that actually there is 'over treatment' in the sense that more people get sent for a CT-scan to be sure. The biosensor therefore is great to potentially avoid unnecessary CT scans as these aren't without risk due to radiation. He mentioned that there aren't really a lot of patients being 'missed' as often, when in doubt, people are sent for a CT scan. Another important note he made was that the biosensor will likely not substitute the current diagnosis but that it will support the decision-making process. He also described clearly what he would look for in a biosensor in regard to how the result is displayed, how quick it should be, where we could use it etcetera. He also provided info on how a biosensor can be used, either as diagnosis or prognosis, and that in each case, the biosensor has to have a slightly different characteristic. For diagnosis you really need a quicker analysis than currently the case, but for prognosis, the measurement is allowed to take a bit longer. Bram Jacobs also was able to give us some information on some more business-oriented questions regarding costs and what parties are interesting to approach when bringing the biosensor on the market. This can be integrated into the business case. We can also incorporate some of his answers in the World Value assignment and out innovation teams is also able to use his answers.

II.IV Employee WUR Value Creation (March 9th 2023)

We had a meeting with Carla van Heck, an employee of the Wageningen University & Research (WUR) in the department of Corporate Value Creation. She is working on spin-offs from WUR and with WUR researchers, mostly about medical products (since patents are most important in this field). Her work includes patents/publications, business models and funding. She explained to us Intellectual Property and want we should keep in mind when publishing or sending information. We also talked about companies that might be interesting for us to contact and about our business case. She recommended us the razor blade model. This would mean we 'sell' a cheaper reader, but for every chip you have to pay. We could also consider leasing the reader. Furthermore, she gave us some tips on when hospitals decide to choose a new diagnostic tool. They usually consider the costs: a new diagnostic tool can be chosen if it is cheaper than the competing diagnostic tool and it should also be cheaper than the treatment. She also gave us some tips for further interviews: we should ask customers how we could help them and if we help them with our invention, how much they are willing to pay for it.

II.V Entrepreneur (March 30th 2023)

We had an interview with Jurre steens, CTO of Scope Biosciences, a company that developed a diagnostic platform using CRISPR-Cas which can detect specific mutations in a genome. The idea to start a company followed from participation in the student competition iGEM for synthetic biology. At first, everybody did everything in the start-up, but now that Scope has grown and has employers, the tasks are clearer. As a CTO, less to no time is spent in the lab and more meeting potential customers and determining R&D directions. It helped at first to focus on the technique and not the application. Thus, at first, the product needs to work before the costs can be lowered. This allowed them to start working with smaller companies in co-development before having huge clients. Consequently, the first field was the detection of plant diseases. They would like to continue in human diagnostics, however, in this field validation is a larger obstacle. Moreover, co-development helped to get the product out, which prevents spending too much time in the lab optimizing. The customers are reached through company visits, pitches and networking, they try to find customers themselves after matching them to a problem. The customers and investors value having clear numbers of what the sensor can reach. Overall, difficulties include running a business, logistics and paperwork/validation. Moreover, applying for a patent is a long and costly process. After 4 years there is still no patent. It helps to surround yourself with experts and like-minded people that are also in the entrepreneurial process so that you can learn from each other. Also, you must persevere through the many highs and lows, it helps to celebrate all successes.

II.VI Expert Point of Care device development (May 12th 2023)

We had a meeting with Arjan Tibbe from the company One Planet. Besides the information provided about our current idea, he also shared his opinions on the idea of the development of a Point of Care sensor. The market for the point of care sensors is very tricky. We can offer, with our idea a faster diagnosis, but is this really necessary? Priority samples that get sent to the lab often have results within an hour or so. Arjan asked us if we think that this really matters for the patient's health. He mentioned that in his opinion, one of the only biosensors that really has a share of the market is the glucose biosensor for diabetic patients.

II.VII General practitioner (June 7th 2023)

We had a meeting with general practitioner Ingrid Hummelen, as a GP you know that at the time you receive the patient, you're most likely not diagnosis the patient at the 'acute' moment. Asking the patient about both broad and in detail about their experience is thus crucial to find out what happened, and what the possible diagnosis can be. The focus is on how the accident happened and which symptoms the patient experiences. Currently the GP does not have quantitative tools to diagnose the patient. The physical examination that the GP could do stays on basic levels, like reflexes of the eye, and arms. But also, blood pressure or heart rhythm could be somewhat of an indication. The GP indicated that the addition of a biosensor could help to bridge the request of an MRI scan by the GP to the specialist. The GP also told us that just a general indication of there being a brain injury could give the patient a certain feeling of confirmation instead of just blindly trusting the subjective indication of a general practitioner and gives the patients a concrete answer to the symptoms they experience.

II.VIII Diagnostic laboratory (June 9th 2023)

During the research to find out what our market would be, and interview with dr. Angela Bikker-Koornneef from the SensUs partner Unilabs was held. Unilabs is a broadly oriented diagnostic laboratory providing their services to general practitioners, and in-house diagnostic laboratories of hospitals. Unilabs does not specifically focus on neurological diagnostics like GFAP measurements, but they do focus on clinical-chemical diagnostics. GFAP would, in this case, fall under this category. Unilabs has experience with PoC biosensors and use them in order to speed up diagnosis. Especially when overcrowding is bound to happen, and not all samples can be measured in time, PoC sensors serve an important role. To their guidelines, PoC biosensors need to be 'idiot-proof'. A device that is easy to interpret and handle are therefore important to take into account during development. Other important factors are portability and storage (cold, or room temperature). Angela advised us on the implementation of our device. Clinical-chemical laboratories decide which PoC testing devices will be available in the hospital. The customer will still include doctors, but with consultation of clinical chemists. It became apparent that insurers do not have a say in which ways of testing will be implemented.

II.IX General practitioner (July 5th 2023)

The interview with Nancy Heijne was our second general practitioner we interviewed. Overall, we have asked similar questions as we did with our first interview with a GP. In response to the possibility of a biosensor coming to the market she was positive. Her opinion was that it would mostly serve as an additional tool. She would still first do the anamnesis and the bodily check-up and then use our biosensor to be absolutely sure. She also mentioned that if our biosensor would get on the market, it would probably first be used by hospitals and only later will it go to the general practitioners. She also mentioned the NHG (Nederlandse Huisartsen Genootschap). This fellowship is an independent group of general practitioners and scientists who decide what protocols and tests are used by the general practitioner. If our biosensor would be approved by them, then the GPs would use them. Overall, Nancy Heijne provided use with valuable information and insights that could be applied to our business case.

III Calculation costs materials per strip

The test strips are prepared by making a lateral flow card, consisting of, transparent backing, nitrocellulose membrane, conjugate pad, sample pad and absorbent pad. The nitrocellulose membrane is sprayed with capture antibodies and the conjugated pad is sprayed with detection antibodies coupled to Europium Nanoparticles. One lateral flow card is cut into approximately 30 test strips. Below calculations of the amounts of materials in one lateral flow card are given, together with the calculated price. To calculate the costs per strip, the costs per card are divided by 30.

- Nitrocellulose: 20 cm x 25 mm of nitrocellulose is needed for one card. A role of 5 m of nitrocellulose costs 250 USD which corresponds to €226. With one role 25 cards can be made, so the nitrocellulose per card costs €9.04, and €0.30 per strip
  (https://www.cytivalifesciences.com/en/us/shop/lab-filtration/immunodiagnostics/lateral-flow-membranes/ff120hp-membranes-p-00760#tech-spec-table een rol is 50 m.).
- Transparent backing: costs €550 per 100 backings, so €5.50 per card and €0.183 per strip

(https://trade.kenoshatapes.com/collections/frontpage/products/lateral-flow-backing-card-bundle-100-cards).

- Absorbent pad: cost €103 per 100 sheets. Per card we use on 1/3rd of a sheet. Per card it costs €0.34 for the absorbent pad and thus €0.01 per strip
  (https://www.sigmaaldrich.id/id_en/cfsp223000-ph).
- Sample pads: cost €96 for 100 sheets. Per card we use 1/3rd of a sheet. Per card it costs €0.32 for the absorbent pad and thus €0.01 per strip
  (https://www.sigmaaldrich.com/NL/en/product/mm/cfsp223000).
- Conjugate pad: cost €179 for 100 sheets. Per card we use 1/3rd of a sheet. Per card it costs €0.60 for the absorbent pad and thus €0.02 per strip
  (https://www.sigmaaldrich.com/NL/en/product/mm/gfcp203000).
- Detection antibodies: the used protocol to couple the EuNP's to the antibodies requires 50 µl EuNP's solution and 120 µg antibody for a final volume of 400 µl of EuNP's coupled antibodies. Per card we spray 400 µl diluted conjugate antibodies coupled to EuNP's. The conjugate antibodies coupled to EuNP's are 50 times diluted, so we use 8 µl undiluted conjugate antibody coupled to EuNP's and thus 2.4 µg antibody. Antibodies were bought for €50 per 1 mg. 2.4 µg antibody corresponds then to a price of €0.12. Per card we use 1 µl EuNP's. For 5 ml EuNP's the price is €780 and a card thus costs €0.16 and a strip €0.005.
- Capture antibodies: per card we spray 40 µl capture antibodies with a concentration of 1500 ng/µl. This corresponds to 6 µg of capture antibodies, antibodies were bought for €50 per 1 mg, which costs €0.30 per card and €0.01 per strip.
- Running buffer: 80 µl running buffer is needed. Buffer is produced in large amounts (liters) and the price of 80 µl buffer is negligible.