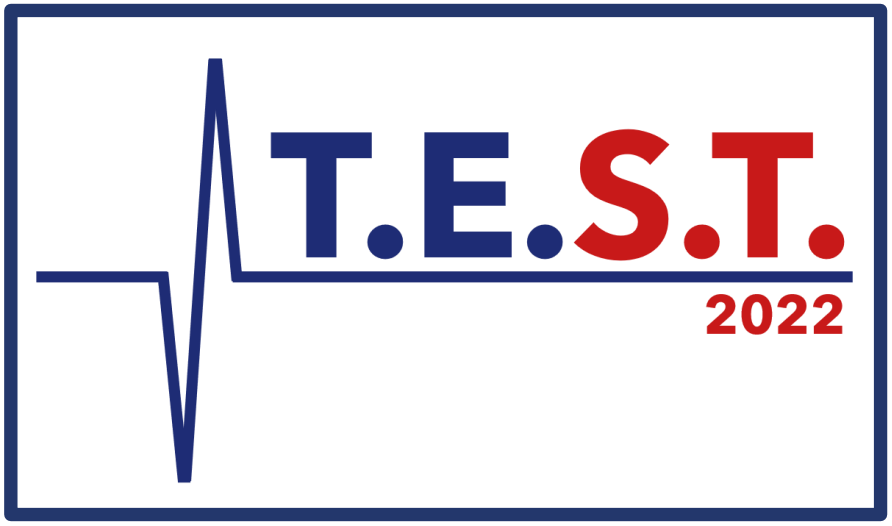
Team Results Document
TU/e Sensing Team (T.E.S.T.)



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SensUs

TU/e EINDHOVEN UNIVERSITY OF TECHNOLOGY

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1. Summary for the SensUs website

T.E.S.T. presents to you an innovative and rapid biosensor for measuring interleukin 6 (IL-6) to detect acute inflammation. The biosensor will detect sepsis in mere minutes, which will allow earlier treatment that reduces the number of patients in the ICU and corresponding high costs, and avoid unnecessary admission of antibiotics. In the biosensor IL-6 is captured between the sensor surface and a magnetic nanoparticle (MNP) with fluorescent dye via a sandwich assay. A laser is aimed on the sensor surface in such a manner that only the fluorescent dye that is on the surface sends out light. The MNP those dyes are bound to also scatter some of the laser light in all directions. This emitted light from both sources is captured and converted to the appropriate IL-6 concentration. MNPs can be pulled using magnets, which is used to magnetically wash the sample by pulling the unbound MNPs from the sensor surface. This way, only the MNPs that have bound IL-6 are measured at the surface. Since, according to healthcare professionals, only measuring IL-6 is not enough to effectively diagnose sepsis, this biosensor concept was developed with the option in mind to measure multiplex, i.e., measuring multiple analytes in one sample.

2. Biosensor system and assay

2.1 Overview

The main principle of the biosensor is a sandwich immunoassay with detection antibodies bound to fluorescently labelled magnetic nanoparticles (MNPs). The use of MNPs allows magnetic manipulation of the particles. This results in a short incubation time in the order of minutes and a low background signal as unbound MNPs are magnetically washed away from the sensor surface. Another benefit employed in the biosensor, is that there are two methods to quantify the analyte of interest: by detecting (1) the fluorescence emitted by the fluorescent labels and (2) the scattered light by the particles. These methods both have advantages: scattering causes a larger signal, while the use of different dyes allows for multiplex measuring. The MNPs are excited by an evanescent wave generated by Total Internal Reflection (TIR) of laser light, which enables local excitation of the MNPs bound to the surface by the analyte. This makes the detection of Interleukin-6 (IL-6) possible.

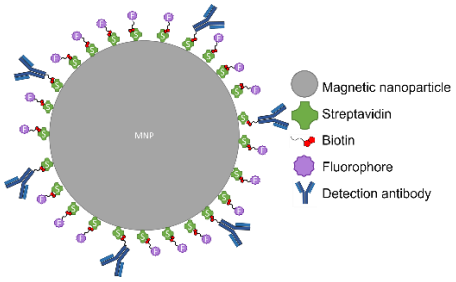

Figure 1: Magnetic nanoparticle coated with streptavidin and functionalized with dAb and fluorescent dye

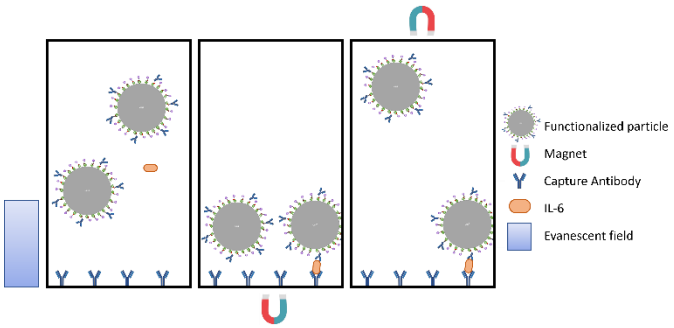

Figure 21: Binding of functionalized magnetic nanoparticles to the cartridge surface with IL-6, with an evanescent field present

2.2 Molecular recognition and assay reagents

MNPs coated with streptavidin are functionalized with detection antibodies (dAbs) and fluorophores as shown in Figure 1. Capture antibodies (cAbs), that bind to a different epitope of IL-6, are immobilized onto the sensor surface through physisorption.

On the sensor surface, a sandwich complex is formed in the presence of IL-6 between the cAbs on the surface and the dAbs on the magnetic beads, as shown in Figure 2. With the help of magnets, the speed of the assay is increased. Besides, it ensures that the detected signal only originates from particles bound to the surface through interactions with IL-6.

Excitation of the MNPs is achieved using an approach comparable to Xiao-Hong [1] through TIR. This technique generates an evanescent wave that decays exponentially, selectively exciting MNPs close to the surface. Therefore, the unbound beads will not contribute to the signal and thus, the signal is dependent on the concentration of IL-6 and the background noise is low.

Dynabeads™ MyOne™ Streptavidin C1 (Thermo Fischer Scientific) are used as magnetic beads. A pair of two monoclonal anti-IL-6 antibodies (L395 and L143, HyTest) is employed to form an IL-6 specific bond between the surface and the beads. L395 is used as the capture antibody. The detection antibody, L143, is functionalized using EZ-link^{TM} NHS-PEG$_4$ biotin (Thermo Fischer Scientific) to allow the functionalization of the magnetic beads through a streptavidin-biotin interaction. Furthermore, the beads are functionalized with Atto 655-biotin dye (Merck). Finally, the beads are incubated with Biotin-mPEG (Nanocs) to suppress nonspecific interactions. After functionalization with cAbs, the cartridge surfaces are incubated with BSA (Merck) to suppress nonspecific interactions with the functionalized beads. The assay is performed in a buffer containing BSA and Tween20 (Merck) to further block nonspecific interactions.

2.3 Physical transduction

As shown in figure 3, a 650 nm laser diode (Thorlabs CPS650F laser diode module) is aimed at a prism to create an evanescent field on the sensor surface, which is used to excite the MNPs near the sensor surface. The emitted light passes through a mask, only allowing light from the sample to pass. A photodetector (Thorlabs PDA36A2, Si Switchable Gain Detector) is used to convert the optical signal into an analog electrical signal. This analog signal is converted to a digital signal using an analog-to-digital converter (ADS1115, 16-bit ADC). Signal processing is performed in a Raspberry Pi 4 to determine the concentration of IL-6.

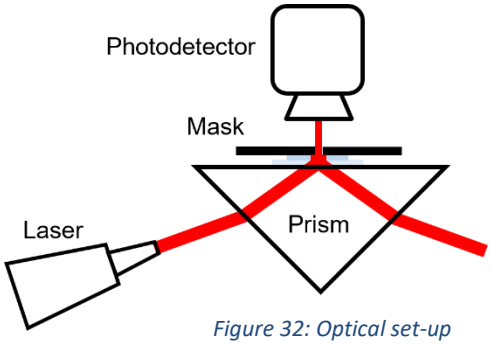

Figure 32: Optical set-up

Cartridge technology

The cartridge consists of a flow cell sticker (SecureSeal™ Hybridization Chambers 9 mm diameter x 0.8 mm height, Grace BioLabs) placed on a glass coverslip (24x40mm, #1.5 optical density, Menzel). The MNP solution and IL-6 sample are mixed outside the cartridge and pipetted into the flow cell, through one of the access ports. Immersion oil (Thorlabs OILCL30, n = 1.518) is applied to the prism to remove air between the prism and the glass slide. This allows for the creation of an evanescent wave at the glass-sample interface.

A key feature of the cartridge technology is the magnetic actuation of MNPs, which is used to speed up the incubation and separation, as described by Bruls et al. [2]. For this purpose, a magnetic actuation stage, separate from the biosensor itself, is developed and can be seen in figure 4. The stage consists of two permanent magnets that can be moved towards and away from the cartridge through the use of a linear actuator, which is powered by a servomotor. To speed up the incubation phase the lower magnet is brought in proximity to the sample which pulls the MNPs towards the functionalized surface. After incubation, the upper magnet is brought near the sample to pull the unbound MNPs away from the evanescent field.

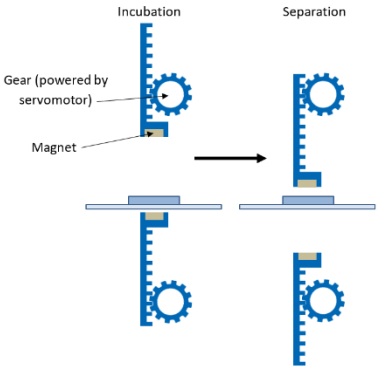

Figure 4: Schematic set-up of magnetic actuation stage

2.1 Reader instrument and user interaction

The ADC is used to read out the voltage output from the photodetector and converts it into a 16-bit signal 1024mV. The Raspberry Pi 4 is used to control the ADC and photodetector, which measure the intensity of the light for 30 seconds, 8 times per second. Then, the average intensity value is calculated and converted to the concentration IL-6 and corresponding class using a previously determined calibration curve. Subsequently, the concentration and class are shown on an LCD touchscreen (7-inch TFT-LCD Display 1024x600 pixels with touchscreen) via a graphical user interface (GUI). All components are powered by a power supply (Mean Well RPT-60B). The dimensions of the biosensor are 188x128x134mm, figure 5 shows the exterior design and further information can be found in appendix 9.1.

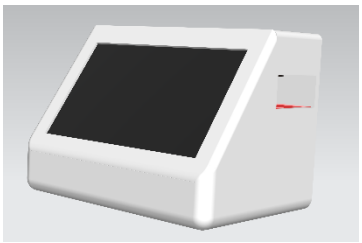

Figure 5: Biosensor design

For each sample, the following six actions are performed. (1) The functionalized MNPs are added and mixed to the plasma outside the cartridge. (2) The sample is pipetted into the cartridge. (3) The cartridge is placed in the magnetic actuation stage. (4) A droplet of immersion oil is applied onto the prism, the cartridge is removed from the magnetic actuation stage, and inserted into the biosensor (5) The measurement is started using the GUI and the concentration of IL-6, as well as the corresponding class, are displayed.

3. Technological feasibility

3.1 Molecular recognition

3.1.1 Specificity

The selected antibody pair, L143 (dAb) and L395 (cAb), is based on the research of Ella Meijer, see appendix 9.2 [3]. It was reported that when using L143 as cAb together with L395 as dAb, the pair gave the highest response in an fBPM experiment, reproduced by the team. To avoid nonspecific binding of MNPs to the surface, multiple reagents are used, as written in chapter 2.1.

3.1.2 Analyte-to-MNP ratio

The analyte-to-MNP ratio was calculated to analyse the linearity behaviour of the sensor. In the cartridge, 40 µL functionalized MNP solution (0.13 pM, see appendix 9.3) and 13 µL sample (maximum of 21 pM after dilution) are added. Thus, the maximum IL-6: MNP ratio is 161 IL-6 per MNP. The ratio of 40 µL MNP solution and 13 µL IL-6 sample was based on a choice to keep the sample dilution to a minimum but to have a sufficient amount of beads to bind IL-6. However, the large number of IL-6 per bead causes nonlinear behaviour of the assay.

3.1.3 Bead functionalization

During functionalization, fluorescent dye (25 µM) and biotinylated dAbs (100 nM) are added in equal volumes to 17.6 pM MNPs. The concentration of dAbs is chosen to avoid clustering of MNPs during the incubation phase. The MNPs have ~20 µg/mg binding sites for antibodies [4], which corresponds to a maximum of 75 thousand binding sites per nanoparticle, see appendix 9.3 as the fluorescent dye molecules are smaller than the antibodies, this is a conservative estimation and there may be more available binding sites.

Assuming the fluorescent dye and antibody bind with similar efficiency, the dAbs occupy around 0.4% of the binding sites, corresponding to 300 dAbs per bead. This means that at the highest concentration of IL-6, approximately 54% of dAbs are occupied.

Experimental data from the team confirmed that the MNP is saturated at a concentration of between 10 and 100 µM, confirming estimations of 25 µM, as shown in appendix 9.4.

3.1.4 Dose-response curve

A dose-response curve is made to evaluate the biosensor. Instead of the magnetic actuation stage, MNPs were sedimented by gravity. The signal that is measured is caused by scattering and fluorescence of the MNPs, as this ensures a better dynamic range of the signal. All measurements were performed in duplication.

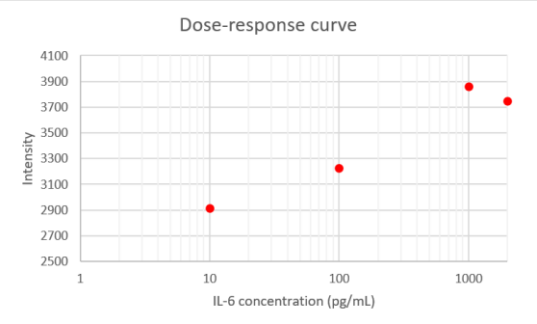

Figure 6: Dose-response curve of four IL-6 concentrations. Mean values of the duplo are plotted.

3.2 Physical transduction

Calculations were made to ascertain whether only the bound MNPs get excited. For generating an evanescent field, the light should approach the glass-plasma interface at an angle larger than the critical angle, which is 63.09° for a transition from glass to plasma (see appendix 9.5 for calculations). The intensity of an evanescent wave decreases with increasing incidence angle, so an incidence angle of 64° was taken (63.09° with safety margin of 0.91°). Furthermore, to validate whether only MNPs close to the sensor surface are excited, the intensity of the evanescent field as a function of depth was calculated. The intensity of the evanescent field decays exponentially with distance and equals 3.8% of the surface intensity at a distance of 1µm from the sensor surface. This indicates that excitation mainly happens within one MNP distance.

As the emitted light travels through the sample from the sensor surface, a part of the emitted light is scattered and/or absorbed. Because no filter is used, autofluorescence of blood plasma is not removed, which results in additional background signal.

3.3 Fluidic cartridge

To ensure that the scattered light detected by the photodetector is only originating from the sample, a black mask was used. This mask covers the entire cartridge, except for a cylindrical area above the sample area, with a diameter of 4 mm.

As magnetic actuation of MNPs is an essential part of the cartridge technology, the velocity and magnetic force were estimated. This was done to estimate the time needed to remove the unbound MNPs from the sensor surface and to ensure that the magnetic force does not break the bonds within the assay. The velocity was estimated to be $7.13 \cdot 10^{-4}$ m/s with a permanent magnet at a distance of 5 mm from the sample. This results in a time of 1.14 s to travel from the sensor surface to the top of the flow cell (see appendix 9.6 for calculations). The magnetic force was estimated to be 9 pN, which is lower than the force typically required to dissociate the bond between an analyte and an antibody (approximately 50 pN). Using these estimations, it was determined to bring the lower magnet 5 mm from the sample for an incubation time in a matter of seconds. Subsequently, the upper magnet is brought 5 mm from the sample for a separation time in an order of seconds.

3.4 Reader instrument

The standard deviation of the sensor was calculated to find the precision of the sensor. Experiments show that the sensor measures a baseline of approximately 510 arbitrary units (a.u.) and a standard deviation of <1%. The signal of an IL-6 range from 10 pg/ml to 2000 pg/ml falls between 3300 and 4000 a.u. with the same standard deviation.

This standard deviation was acquired by averaging the signal over a 30 second measurement. The SNR can be improved by a factor square root of the amount of measurements [5]. This relation was used to determine that the rate at which the SNR improves slowly nears an asymptote after 30 seconds, meaning measuring longer does not reduce noise as much as it costs valuable time.

3.5 Future improvements

The magnetic actuation stage, as described in paragraph 3.3, has not been realized at time of writing yet. This will be done in the coming weeks.

In the current hardware setup, the signal coming from the fluorescent dye is not strong enough in relation to the noise to accurately measure the IL-6 concentration. A more sensitive photodiode, for example an avalanche photodiode (Thorlabs), could be a solution to make measurement of low fluorescence possible.

It is important to note that measuring fluorescence enables the extension to a multiplex biosensor. Future development should focus on the incorporation of sets of MNPs coated with different fluorescent dyes and antibody sets targeting different molecules involved in sepsis, like CRP [6], CTP [7]. The Super Bright dyes (ThermoFischer) can all be excited at the same wavelength, but emit a different wavelength compared to each other, see appendix 9.7. This would also involve a revolving filter wheel to measure multiple analytes consecutively. This way, only one laser and one photodiode are needed, but multiple tests could be run back-to-back with the same sample.

Furthermore, to decrease the number of steps in sample handling, the magnetic actuation stage should be integrated in the biosensor.

The sensor does not exhibit linear behaviour. It is expected that to improve the linearity of the sensor, the concentration of MNPs will have to be larger. For linear behaviour, it is required that only one IL-6 will bind to one MNP, which is most easily achieved by having a large amount of MNPs. The concentration would have to be a factor of 161 higher than in the current sensor, which is not achievable with the current MNP stock solution. As the ratio between the concentration of analyte and bead is essential for the performance of the assay, this may still be improved in the following weeks.

In order to increase the transportability and ease of use, it is possible to store functionalized MNPs in the cartridge using sugar drying. This would lengthen the expiration time of the functionalized surface. It would also remove a step in the sample handling, because there is no longer a need to add the particles to the sample outside of the cartridge.

4. Originality

4.1 Team captain

After broad research on the field of biosensing, the team initially decided to develop a sensor based on plasmon enhanced fluorescence [8]. The idea was to combine gold nanorods (AuNRs) coated with fluorescent dye as ultrabright labels with MNPs. However, after a couple of experiments, it became clear this was not a viable option for the competition, due to time constraints and difficulties functionalizing AuNRs. To simplify the concept, the AuNRs were removed and the team focused on MNPs. After several interviews with medical [6][7][9][10] and biosensing [11] professionals, the merit and necessity of a multiplex sensor, especially in the case of diagnosing acute inflammation in elderly (age >65), became clear. At the moment, IL-6 is not used as a biomarker for sepsis by medical professionals. Therefore, combining IL-6 as a biomarker together with ones that are already used, improves the diagnostic value of the biosensor. With some adjustments and selecting an appropriate dye (see appendix 9.4), the principle was functional. This was thought of completely by the team itself. It was later experimentally found that measuring scattering instead of fluorescence yielded higher signals, making it a more viable option for the competition due to developmental time constraints. However, further development could still make fluorescence measuring and multiplex assay development possible.

At first, the optical set-up excited both the bound and unbound MNPs, which was detrimental to the specificity. When the team came across the phenomenon of evanescent fields, they realized it was the perfect solution since only the bound particles at the surface get excited.

The usage of MNPs enables magnetic separation and, together with TIR, increases the signal-to-noise ratio. The idea of magnetic separation was inspired by the paper of Bruls et al. [2], which was later developed further by Philips as the Minicare I-20 [12]. The idea of magnetic separation was further developed for our sensor with the help of our supervisors. The magnetic actuation stage itself was developed independently by the team.

The first steps for the electrical circuit were made with help from a T.E.S.T. alumnus, who we consulted multiple times during the process. The sensor design and the optical setup were developed entirely by the team itself. The final biosensor was constructed entirely by the team itself.

4.2 Team's supervisor

The team started with a literature search and decided to focus on detection technologies that could be supported by the research groups at TU/e. After many brainstorms, a sensor concept based on the enhanced fluorescence from dyes in the vicinity of gold nanorods emerged after discussions with researchers at TU/e. Although the students used available concepts, they designed the assay based on their own understanding of sandwich assays. Unfortunately, the complexity of the chemical functionalization and the required short time to result for the sensor turned out to be unsurmountable. The switch to fluorescent magnetic particles was decided by the team members independently (thereby sticking to fluorescent detection) and was primarily motivated by the easy combination with magnetic actuation (short time to result) and the time pressure to be able to have a sensor ready at the SensUs testing event. The readout unit was designed and constructed by the team from scratch.

Signatures

Team captain Naud van Rosmalen		Vice-captain Bram Boerenkamp	
Supervisor dr. L. J. (Leo) van IJzendoorn		Coach ir. B.A.K.C. (Chris) Vu	

5. Translation potential (max. 3 A4)

5.1 Business model canvas

<table>
<tr>
<td rowspan="2">Key Partners
Eindhoven University of Technology
Future Diagnostics
Qarad
V.O. Patents
Medical federations
Patient associations
National governments
Insurance companies</td>
<td>Key Activities
Sensor and assay development
Acquire and maintain medical license and patent
Gathering funds</td>
<td rowspan="2">Value Proposition
Our biosensor will detect sepsis in mere minutes, which will allow earlier treatment that reduces the number of patients in the ICU and corresponding high costs and avoid unnecessary admission of antibiotics.</td>
<td>Customer Relationships
Performing studies of the efficacy and generating feedback from the GPs about the use of the biosensor in their workflow</td>
<td rowspan="2">Customer Segments
GP’s offices
Intensive care units
Post-operative patients
Ambulances
First Aid (emergency room)
3rd world countries</td>
</tr>
<tr>
<td>Key Resources
- A dedicated team of 9 students
- Scientific advisors
- Lab facilities
- Medical license and patent</td>
<td>Channels
GP’s offices
Insurance companies
Distributions companies</td>
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Medical License & Patent
Marketing
Rent
Quality assurance
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Cartridge production 1.50 EUR</td>
<td colspan="2">Revenue Structure
Sensor sales 2000 EUR
Cartridge sales 15 EUR
Grants & Investments</td>
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</table>

5.2 Stakeholder desirability

In 2021 sepsis affected around 50 million people and killed nearly 11 million. The fact that sepsis causes suffering on this scale is tragic. However, the knowledge that sepsis is effectively treatable in early stages makes it even more heart-wrenching. The urgency of treatment is illustrated by the fact that a delay of 1 hour in antibiotic delivery raises the chance of mortality by 7.6 percent for septic patients with hypotension [13][14]. Moreover, many surviving patients deal with the consequences of sepsis for the rest of their life. Patients with late-stage sepsis have to be admitted to intensive care units for an average amount of 17 days. Sepsis treatment in the intensive care unit (ICU) costs 1300 EUR per day in the Netherlands [15]. Furthermore, lowering hospital bed occupancy is important, particularly in the context of the recent pandemic. In the Netherlands roughly 35 thousand individuals have been diagnosed with sepsis in 2020, with 10 000 patients being admitted to the ICU [16].

The size of the global sepsis diagnostics market was 615 million USD in 2021. The market can be divided in laboratory (85%) and point-of-care (POC) applications (15%). The total market is expected to grow with compound annual growth rate (CAGR) 9,6% in the period 2022-2030 [17]. The laboratory segment is expected to grow at a higher rate than POC segment [17]. The CAGR of the POC segment is assumed to be 5%. The European market size is approximately 7.8 million USD in 2027 calculated in appendix 9.8. Within this market several patient groups can be identified. The patient groups that are admitted to the hospital (ambulances, post-surgical, Emergency Department, Rapid Response System) are already in a later and more severe stage of sepsis and demanding rapid antibiotic treatment [21][22]. A diagnostic biosensor adds no value to these scenarios. When it comes to sepsis in developing nations, the solution is far more complex than just adding a biosensor. Increased awareness, illness surveillance, and enhanced hygiene for example, are especially important here [23], as can be seen in appendix 9.9. The patient group that is most attractive for T.E.S.T.’s biosensor are those coming to the GP with septic symptoms.

Sepsis is more common among the elderly, and with an aging population this risk group is expected to expand in the coming years [18]. The average age of Dutch septic patients admitted to the ICU in 2017 was 67 [19]. This group has a higher risk of permanent organ damage and death. This makes it crucial to stop sepsis at the earliest stage possible. However, early symptoms in elderly patients are more difficult to distinguish from regular flu or bladder infections [20][9]. According to new research, 48 % of patients admitted to the ICU with sepsis had previously contacted their general practitioner (GP). However, in 43% of these admitted patients, the GP failed to detect an infection. In these cases, mortality rates were nearly three times higher than in the patients whose GP identified infection during the initial consultation. [22] After different interviews with medical professionals and literature research on the market size, T.E.S.T.’s biosensor is the most attractive for the elderly patient group at the GP (Appendix 9.9).

Therefore, T.E.S.T.'s biosensor is intended to be used during consultations of elderly patients at the GP office. The biosensor will give an objective measurement based on which the GP will decide on further care, which ranges from direct referral to the ICU, antibiotics at home or no treatment. Hence, use of the biosensor avoids unnecessary hospitalization, reduces antibiotic prescriptions and reduces high health-care costs. Additionally, it allows sick elderly patients to recover at home with antibiotic treatment, which improves their quality of life. It is expected that T.E.S.T.'s biosensor will lead to approximately 6% fewer septic patients being admitted to the ICU annually. This results in 50 million EUR less costs for the healthcare system annually, as is calculated in Appendix 9.10. Early detection and treatment results in both fewer days spend in the ICU as well as less long-term care for septic patients.

In order to illustrate the interactions between the T.E.S.T.'s biosensor and its users, the following customer journey is made, based on the patient journey of a real case in the office of GP Corrie Jongsma (Appendix 9.11). "A patient of 86 years old, came to the GP with flu symptoms and body temperature of 39 Celsius degrees on Friday. The diagnosis of sepsis, due to an inflammation on the heart valve, was established on Sunday. Unfortunately, the heart valve was too badly damaged by that time." If T.E.S.T.'s biosensor would have been used during the doctor's visit on Friday, and irreparable damage to the heart value could have been prevented. The biosensor will be used by the GP or their assistant using blood from a finger prick, after which the results will be available in 5-10 minutes. Because the test is performed by a GP, cartridges can be disposed of safely with other medical waste. The sensor is easily integrated in the workflow of the GP as they already use similar point-of-care devices [6][7][8][9][24]. Moreover, T.E.S.T. will stay in close contact with the GPs to validate and improve the user friendliness of the sensor.

Nowadays the GP uses a C-reactive protein (CRP) rapid point-of-care test to detect infection and sepsis [8][24]. In the Netherlands these rapid tests have been supplied for free to the GPs by the laboratories. Afterwards the laboratory claims a price of 4.62 EUR per test and 9 EUR for order costs per test (to cover reagents, controls, equipment and staffing) from the health insurers. Next, the GP claims a second standard consult (20 minutes), if performing the test takes more time than one consult [24]. The marker CRP is less reliable for detecting sepsis, since elevated CRP levels are not always seen in sepsis, which might lead to a false-negative result [6]. In comparison to CRP, IL-6 concentrations are elevated and measurable in the blood at an earlier time. Nevertheless, the IL-6 biomarker is a generic biomarker for inflammation [6][7]. Therefore, T.E.S.T. aims to expand the biosensor into a multiplex assay, allowing it to assess several biomarkers (IL-6 together with CTP and CRP) for sepsis simultaneously [6][7][10]. Several biomarkers, each corresponding to a separate fluorescent signal, will be identified simultaneously within the same sample by using different light filters in the detection set-up of the sensor.

5.3 Business feasibility

T.E.S.T. aims to reduce the number of patients with sepsis at the ICU by widespread use of its biosensor. Before the sensor can be brought to the market, the sensor and assay have to be developed further. In addition, a medical certificate and patent must be obtained before market entry in 2027

In 2023 and 2024 the focus will be on research and development. Cartridge handling will be minimized to increase reproducibility. The current IL-6 sensor will be developed into a multiplex sensor, to increase the accuracy of the diagnosis of acute inflammation. In cooperation with infection disease specialists the additional biomarkers will be chosen, such as CRP and CTP [6][7]. The signal-to-noise ratio of the biosensor can be improved through research with Eindhoven University of Technology. Cooperation with Future Diagnostics, who specialize in the production of in vitro diagnostics, will make realisation of aforementioned development goals achievable.

Additional research will be carried out to assure a robust sensor performance for all reasonably expected circumstances. For example, the biosensor must work under slightly different temperatures and must be able to resist small shocks as it is meant to be portable, for home visits of the GP.

During the R&D phase the foundation for a successful medical license application has to be laid. The regulatory burden for CE certification of an in vitro diagnostic device is high [26]. As T.E.S.T lacks specialization in this field the application will be mediated by a company specialized in licensing medical devices, for example

Qarad which is part of the QbD group. The application has to be submitted in an early phase since the process takes years and there are significant waiting times for review by notified bodies [26]. In tandem, a patent application will be made with the help of a patent attorney firm such as V.O. patents [27].

In 2025 and 2026 a pilot study will be conducted in the Netherlands. Across the entire country GP offices will be invited to participate, to ensure a broad testing group of different socioeconomic and ethnic background. The pilot study will be an appraisal of the added value the biosensor gives as well as an opportunity to get feedback from GP's. During the pilot the upscaling of sensor and cartridge production will be prepared. Custom components will be produced reducing costs of the most expensive parts of the prototype. The production and assembly will be outsourced. The cartridges production can be improved using injection moulding. [34]

The target is market deployment in Europe at the start of 2027. Steps for marketing will be made in the years prior to 2027. The performance of the biosensor and costs saved in the healthcare system will be presented at medical conferences. Connections with insurance companies and medical federations will be made by T.E.S.T.'s in house marketing department. The saved ICU costs will be leveraged to get use of the sensor covered under insurance. Through medical federations, national government and patient organizations T.E.S.T.'s sensor will be promoted. In appendix 9.12 a SWOT- analysis has been added, which gives an overview of the team's strengths and weakness for defining the best marketing strategy for T.E.S.T.

5.4 Financial viability

The current cost for materials of one biosensor is 950 EUR (Appendix 9.13). Combined the photodiode, filter and laser contribute over 75% to this. Through cooperation with an original equipment manufacturer a significant decrease in production cost is deemed reasonable. The production cost of the sensor on a large scale is assumed to be 500 EUR. The price point of the sensor will be 2000 EUR. The current production cost of the cartridge is 1,50 EUR. (Appendix 9.13) Through research into different production methods and optimizing the assay this is expected to be reduced. However, expansion into multiplex will increase the price. In total the costs for production per multiplex cartridge is assumed to be 5 EUR. The price point for a single cartridge will be 15 EUR. This is a bit higher than comparable single marker tests [24]. The GP can get the cost of this test at least partially reimbursed from insurance companies.

For the successful deployment of the biosensor, funding is needed to finance R&D, licencing and marketing. The total development costs for Class II medical devices range from 2.4 to 6 million USD [35]. Because T.E.S.T. has connections with specialists in the field, like the Eindhoven University of Technology and Future Diagnostics, these costs are expected to be on the low end [10].

Application for a medical license including the required clinical trials is estimated to cost at least 150 thousand USD [35]. In this case the costs are higher because application will be outsourced. The costs for patent application in the EU is around 20 000 EUR. This will be outsourced to V.O. patents. After obtaining the patent and CE certification, annual costs are associated to maintain these [26][27].

TEST's biosensor market size is based on the CRP POC market because of incorporation of markers like CRP. That market is at least twenty times larger than solely the POC sepsis diagnostic market based on a bottom-up approach. It has been estimated that T.E.S.T. will be used in one thousand GP offices in 2027, and selling 100 tests per year, per GP office. Hence a total of one hundred thousand tests and thousand sensors will be sold in Europe during 2027, see Appendix 9.14 [24]. Eventually the market size of T.E.S.T.'s biosensor will be expected to grow up to 1% in Europe in 2030, due to the multiplex system that makes it more attractive to use for diagnosing acute inflammation as it will partially replace current POC CRP testing. With current estimations of costs and revenues it is expected that T.E.S.T. will start being profitable in the first year of entering the European market as can be seen in appendix 9.15. Deviation of sensor sales has direct impact on all the next years as we expect that all sensors will be in full use until 2030. This in combination with the disparity between the two methods for calculating market size has a large impact on the time until profit. However, it is still the case that a multiplex POC biosensor for acute inflammation has the potential to lead to profit, especially when considering expanding either globally or toward a variety of biomarkers and diseases.

6. Team and support

6.1 Contribution of the team members

For the development of the biosensor, the team was divided in two sub-teams: sub-team assay and sub-team detection. The assay team was responsible for the development of the biochemical assay and the detection team for the detection set-up of the biosensor. Furthermore, the team had a sub-team responsible for Translational Potential, where they focused on the business model of the biosensor. To function well as a team, organizational tasks were divided within the team: team captain, vice-captain, secretary, treasurer, external relations, public relations and social media.

Bram Arts	Treasurer of the team and member of assay sub-team
Bram Boerenkamp	Vice-captain of the team, member of assay sub-team and Translational Potential
Dennis Brink	Member of detection sub-team and Translational Potential
Lars Daenen	Member of detection sub-team, and responsible for public relations and social media
Manon Holsappel	Member of assay sub-team and responsible for external relations
Naud van Rosmalen	Team captain and member of detection sub-team
Britte Treure	Secretary of the team and member of assay sub-team
Laurian de Vries	Member of assay sub-team and Translational Potential
Marcus Vroemen	Member of detection sub-team, and responsible for public relations and social media

6.2 People who have given support

Our main supervisor dr. **Leo van IJzendoorn** guided the team throughout the whole competition during weekly meetings. Ir. **Chris Vu** provided support during the weekly meetings and assisted the assay team by checking lab protocols. **Claudia Schot** provided support during the weekly meetings and was our contact person for lab-related questions and orders.

Willem Rovers provided materials and room to work in for the detection team. **Ivar de Vries**, M.Sc provided continuous advice to the detection team about the hardware and electronics. The assay team was advised by members of the Molecular Biosensing research group (Eindhoven University of Technology): dr. **Peter Zijlstra**, dr. **Yuyang Wang**, M.Sc, dr. ir. **Arthur de Jong**, dr. **Mathias Dolci**, **Claire Michielsen**, M.Sc, **Ana Ortiz Perez**, M.Sc, ir. **Max Bergkamp**, **Livio Oliveira de Miranda** M.Sc, **Roy Teeuwen**, M.Sc and **Sebastian Cajigas Bastidas**, M.Sc. Dr. **Dave Dekkers** & dr. **Ernst Lindhout** of Future Diagnostics gave us valuable feedback on our assay and detection principle, as well as translational potential.

Medical specialists, prof. dr. **Volkher Scharnhorst** (clinical scientist of the Catharina Hospital, in Eindhoven), Dr. **Remco Dubbeling** (general practitioner at *the GP office Nijst* in Voorschoten), Dr. **Corrie Jongsma** (general practitioner at *the GP office Jongsma and Ten Dame* in Drachten) and Dr. **Elmer Hoekstra** (gastroenterologist of *the Haga Hospital*) provided valuable information regarding translational potential.

Furthermore, **Mark van Hattum**, **Henrike Hartemink**, M.Sc, **Hidde Douna**, PhD and **Michelle van der Heijden**, BSc of Novartis, Dr. Ir. **Rogier Receveur** of Medtronic, **Sascha Massop**, MSc & **Hanka Schlorova** of ThermoFisher Scientific, **Daan Wouters**, PhD & **Marli de Lange**, MSc of VO Patents & Trademarks and **Predrag Tasic**, PDEng & **Luuk Olijve**, PhD of Organon assisted with the Translation Potential.

6.3 Sponsors

The **Universiteitsfonds** of Eindhoven University of Technology provided financial support. **TU/e Innovation Space** provided financial support, advice to the detection sub-team and office space. Furthermore, they organized the TU/e contest during which valuable information for Translational Potential was gained. **HyTest** provided in-kind sponsoring of antibodies and IL-6 via the SensUs Competition.

7. Final Remarks

During the SensUs Competition we learned that the translation of theoretical ideas into a practical solution is accompanied by a legion of unforeseen problems. A systematic approach actually leads to better results or at least a better understanding of what is going wrong. Especially the concept of “one variable at a time” was one of the things we learned the hard way. During conversations with healthcare workers, we also learned the importance of validating everything

The short but valuable time developing our sensor and its accompanying business concept awakened a newfound appreciation of any working point of care sensor. The amount of effort, insight and trial and error to produce a market viable product is larger than expected.

We would like to end by thanking everyone who supported us, especially our supervisor Leo van IJzendoorn and coaches Chris Vu and Claudia Schot.

8. References

1. Xiao-Hong, Z., Lan-Hua, L., Wei-Qi, X., Bao-Dong, S., Jian-Wu, S., Miao, H., & Han-Chang, S., *A reusable evanescent wave immunosensor for highly sensitive detection of bisphenol A in water samples*. Scientific Reports*, 2014,* https://doi.org/10.1038/srep0457
2. Bruls, D. M., Evers, T. H., Kahlman, J. A. H., Van Lankvelt, P. J. W., Ovsyanko, M., Pelssers, E. G. M., Schleipen, J. J. H. B., De Theije, F. K., Verschuren, C. A., Van Der Wijk, T., Van Zon, J. B. A., Dittmer, W. U., Immink, A. H. J., Nieuwenhuis, J. H., & Prins, M. W. J. *Rapid integrated biosensor for multiplexed immunoassays based on actuated magnetic nanoparticles*. Lab on a Chip, 2009, https://doi.org/10.1039/b913960e
3. Meijer, E., *IL-6 antibody affinity screening with a particle mobility assay*, unpublished, 2022
4. ThermoFisher, *Dynabeads™ MyOne™ Streptavidin C1*. Retrieved from https://www.thermofisher.com/order/catalog/product/65001
5. Tkachenko, N.V., *Chapter 7 – Flash-photolysis*, Optical Spectroscopy, 2006. Retrieved from https://www.sciencedirect.com/science/article/pii/B9780444521262500319
6. prof. dr. Scharnhost, V. (clinical scientist of the Catharina Hospital), personal interview, 20-04-2022
7. dr. Hoekstra, E. (gastroenterologist of the Haga Hospital), personal interview, 02-05-2022
8. Li, J. F., Li, C. Y., & Aroca, R. F. *Plasmon-enhanced fluorescence spectroscopy*. Chemical Society Reviews, 2017, https://doi.org/10.1039/c7cs00169j
9. dr. Jongsma, C. (general practitioner in Drachten), personal interview, 06-07-2022
10. dr. Dubbeling, R. (general practitioner in the Hague), personal interview, 01-06-2022
11. Lindhout, E. (Head of Research & Science at Future Diagnostics), Dekkers, D. (Technical architect at Future Diagnostics), personal interview, 15-4-2022
12. Philips, *Leading change in Point-of-Care Testing*. Retrieved from https://www.philips.com/a-w/about/news/archive/blogs/innovation-matters/leading-change-in-point-of-care-testing.html
13. Beckman Coulter, *Early Sepsis Detection.* n.d. Retrieved from https://www.beckmancoulter.com/products/hematology/early-sepsis-detection
14. Markets and markets, *Sepsis Diagnostics Market*, 2022. Retrieved from https://www.marketsandmarkets.com/Market-Reports/sepsis-diagnostic-market-92673155.html
15. Siemens, *IL-6 and LBP: Detection of Infection, Inflammation, and Sepsis*. Retrieved from https://cdn0.scrvt.com/39b415fb07de4d9656c7b516d8e2d907/1800000000046197/b03a994c62ee/0701667_il-6_sepsis_v11-00046197_1800000000046197.pdf
16. Sepsis en daarna, *Feiten over sepsis*, n.d. Retrieved from https://www.sepsis-en-daarna.nl/alles-over-sepsis/feiten-en-vragen/feiten-over-sepsis/
17. Grand View Research, *Sepsis Diagnostics Market*, n.d. Retrieved from https://www.grandviewresearch.com/industry-analysis/sepsis-diagnostics-market#
18. CBS, *Ouderen*, n.d. Retrieved from https://www.cbs.nl/nl-nl/visualisaties/dashboard-bevolking/leeftijd/ouderen
19. Federatie Medisch Specialisten, *Sepsis fase 1*, 2020. Retrieved from https://richtlijnendatabase.nl/richtlijn/sepsis_fase_1/startpagina_-_sepsis_fase_1.html
20. Loots, F., Prins, A., Tjan, D., Koekkoek, K., Hopstaken, RM, Giesen, P., *Sepsis herkennen bij een volwassene*. Huisarts en Wetenschap, 2017, Retrieved from https://www.henw.org/artikelen/sepsis-herkennen-bij-een-volwassene#
21. Ju, T., Al-Mashat, M., Rivas, L., & Sarani, B. *Sepsis Rapid Response Teams*. Critical Care Clinics, 2018, https://doi.org/10.1016/J.CCC.2017.12.004
22. Loots, F. J., Smits, M., van Steensel, C., Giesen, P., Hopstaken, R. M., & van Zanten, A. R. H. *Management of sepsis in out-of-hours primary care: a retrospective study of patients admitted to the intensive care unit*. BMJ Open, 2018, https://doi.org/10.1136/BMJOPEN-2018-022832

23. Heneine, E., Nsutebu, E., Rylance, J., Jacob, S., *A call to action: Sepsis is Africa's Neglected Silent Killer.* African Institute for Development Policy, 2019. Retrieved from https://www.afidep.org/publication/a-call-to-action-sepsis-is-africas-neglected-silent-killer/
24. Ďurč P, Foret F, Kubáň P. *Fast blood plasma separation device for point-of-care applications*. Talant, 2018, doi: 10.1016/j.talanta.2018.02.004
25. European Commission, *New Regulations*, n.d. Retrieved from https://health.ec.europa.eu/medical-devices-sector/new-regulations_en
26. Michelle van de Heijden, BSc (Regulatory Affairs Business Excellence Manager of Novartis), personal interview, 02-06-2022
27. Daan Wouters, PhD (European and Dutch Patent Attorney) and Marli de Lange (Trainee Patent Attorney) of V.O. Patents and trademarks, personal interview 17-05-2022
28. Hopstaken, RM, Verdijk, N., van den Broek, N., Verspaandonk, K., Meulepas, MA, Helder, C., Keyzer, J. *CRP-sneltest in de dagelijkse praktijk.* Huisarts en Wetenschap, 2012. Retrieved from https://www.henw.org/artikelen/crp-sneltest-de-dagelijkse-praktijk#:~:text=Vergoedingen%20vari%C3%ABren%20van%203%2C50,en%20kwaliteitscontrolesystemen%20nog%20niet%20vergoed
29. Martin-Fernandez, M. L., Tynan, C. J., & Webb, S. E. D., *A "pocket guide" to total internal reflection fluorescence*. Journal of Microscopy, 2013, https://doi.org/10.1111/jmi.12070
30. Davidson, M. W., & Fellers, T. J., *Coverslip Correction.* MicroscopyU, n.d., Retrieved from https://www.microscopyu.com/microscopy-basics/coverslip-correction
31. Reddy, N. M., Kothandan, D., Lingam, Chandra, S., & Ahmad, A., *A study on Refractive index of plasma of blood of patients suffering from Tuberculosis*. International Journal of Innovative Technology and Creative Engineering, 2012, www.ijitce.co.uk
32. Thermofisher, *Super Bright Polymer Dyes,* n.d. Retrieved from https://www.thermofisher.com/nl/en/home/life-science/cell-analysis/flow-cytometry/super-bright-polymer-dyes.html
33. ThermoFisher, *Dynabeads Cell Isolation and Expansion Support—Getting Started*. Retrieved from https://www.thermofisher.com/nl/en/home/technical-resources/technical-reference-library/cell-culture-support-center/dynabeads-cell-isolation-expansion-support/dynabeads-cell-isolation-expansion-support-getting-started.html
34. Kauffer, P. H. *Injection Molding: Process, Design, & applications*. New York, United States: Macmillan Publishers, 2011
35. Drlik, M. *How Much Does it Cost to Develop a Medical Device?.* Starfish Medical, 2020

9. Appendix

9.1 Sensor design and components

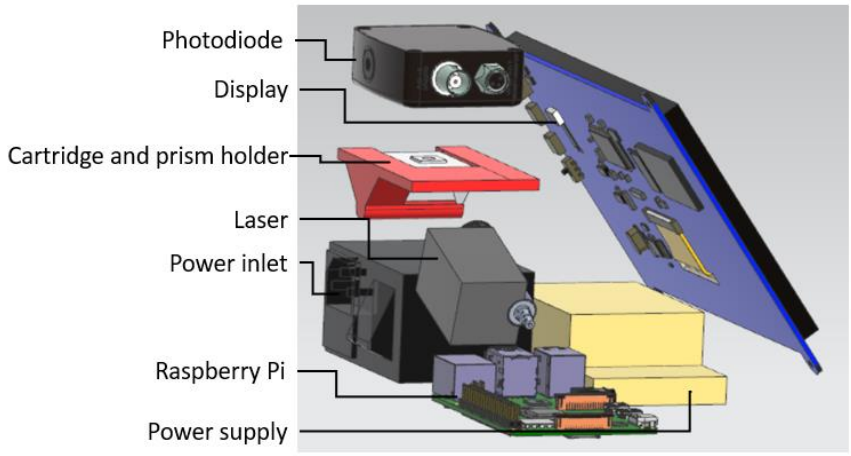

Figure 7: Sensor components

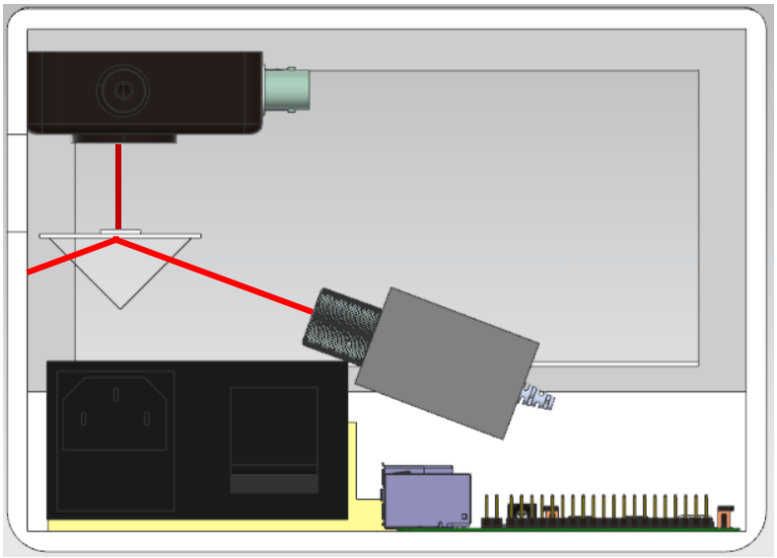

Figure 8: Optical setup in the sensor (without mask)

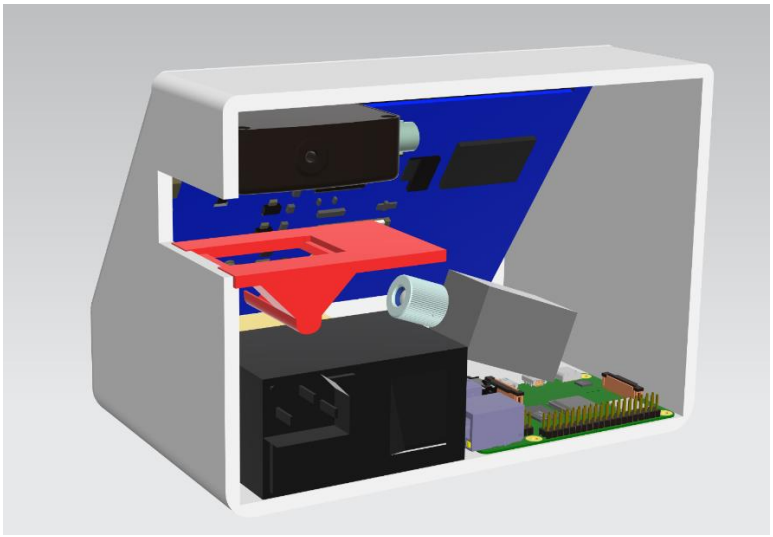

Figure 9: Sensor components in the casing

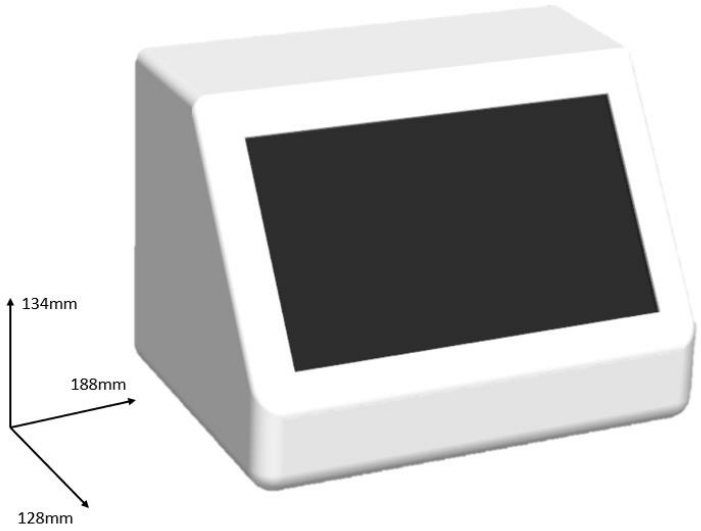

Figure 10: Sensor casing and dimensions

9.2 Antibody pair

In order to decide on a capture-detection pair, data from Ella's report is used [3]. Figure 11 shows that the most sensitive antibody pair is L395 as the capture antibody and L143 as the detection antibody.

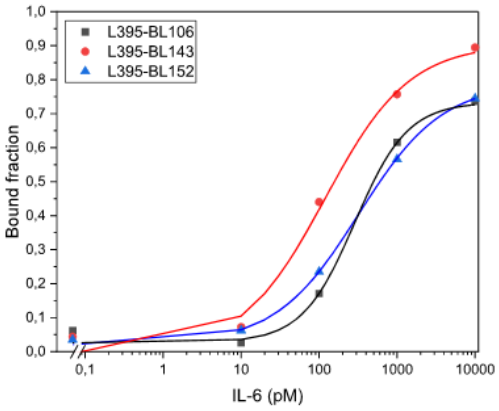

Figure 11: antibody pair comparison.

9.3 Calculations beads

In order to determine the concentration of MNPs in the sensor the following known values are used:

Density MNP's: $1.8\ \frac{g}{cm^3} = 1.8 * 10^6\ \frac{g}{m^3}$ [29]

Concentration MNP stock: $10\ \frac{mg}{ml} = 10 * 10^3\ \frac{g}{m^3}$

Diameter MNP's: $1\ \mu m$

Avogadro constant: $6.022 * 10^{23}$

The volume and weight of a single MNP are used to convert the known concentration into an amount of particles, which can be converted to a concentration using the Avogadro constant. The 100x dilution during preparation is taken into account as well.

$$V_{per\ bead} = \frac{4}{3}\pi r^3 = \frac{4}{3}\pi * 0.5^2 = 0.524\ \mu m^3 = 0.524 * 10^{-18}\ m^3$$

$$m_{per\ bead} = 1.8 * 10^6 * 0.524 * 10^{-18} = 9.432 * 10^{-13}\ \frac{g}{bead}$$

$$\frac{10 * 10^3}{9.432 * 10^{-13}} = 1.060 * 10^{16}\ \frac{beads}{m^3} = 1.060 * 10^{13}\ \frac{beads}{l}$$

$$[beads] = \frac{1.060 * 10^{13}}{6.022 * 10^{23}} = 1.760 * 10^{-11}\ \frac{mol}{l} = 17.60\ pM$$

$$[beads]_{final} = 17.60 * 0.01 = 0.176\ pM$$

The lower bound of the amount of reagents per MNP is calculated using the fill level for antibodies, which is reported by ThermoFisher to be $20\ \frac{\mu g\ Ab}{mg\ bead}$, which corresponds to $133\ \frac{pmol\ Ab}{mg\ bead}$ for a mass of 150 kDa.

$$133 * 9.432 * 10^{-10} = 0.125 * 10^{-6}\ \frac{pmol}{bead}$$

$$1.25 * 10^{-19} * 6.022 * 10^{23} = 75 * 10^3\ \frac{Ab}{bead}$$

In reality, a part of the MNP will be filled by dye and another part by dAbs. If the MNP is filled in the same ratio dye/dAbs as the ratio in their concentrations, 0,4% of the MNP is filled using dAbs, which is 300 spaces. This is higher than the 161 IL-6 per bead at the maximum concentration, meaning that the beads will not be saturated with IL-6 during the experiment, which could cause a maximum detection limit.

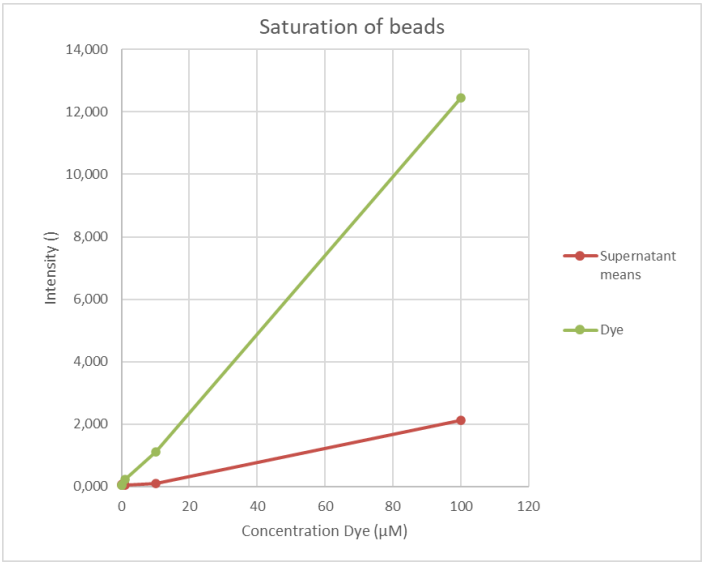

Figure 12: dye saturation.

9.4 Well plate experiment

An experiment was performed to determine the amount of dye required to saturate the MNPs. After MNPs were incubated with dye, the MNPs were washed. The supernatant was stored, which was used to determine the amount of unbound dye. If there was a significant amount of fluorescence in the supernatant, the beads were saturated. As can be seen in figure 12, the beads became saturated at a concentration somewhere between 10 and 100 µM.

9.5 Calculations and simulations evanescent field

The critical angle is calculated using the formula $\theta_c = \sin^{-1}\frac{n_2}{n_1}$ [25]
where n_2 and n_1 are the refractive indices of blood plasma and glass respectively.
Using values of n_2 = 1.351 [26] and n_1 = 1.515 [27] the critical angle equals $\theta_c = \sin^{-1}\frac{1.351}{1.515} = 63.09°$

The intensity of the evanescent wave is calculated using the formula $I = I_0 e^{-\frac{z}{d}}$
where d is the penetration depth $d = \frac{\lambda_0}{4\pi}(n_1^2 \sin^2\theta - n_2^2)^{-\frac{1}{2}}$ [25]
Using $\lambda_0 = 655$nm, $n_2 = 1.351$, $n_1 = 1.515$ and $\theta = 64$, the penetration depth equals
$d = \frac{655}{4\pi}(1.515^2 \sin^2 64° - 1.351^2)^{-\frac{1}{2}} = 306$ nm
Using $d = 306$ nm the intensity at z = 1 µm equals $I = I_0 e^{-\frac{z}{d}} = I_0 e^{-\frac{1000}{306}} = 0.038 I_0$

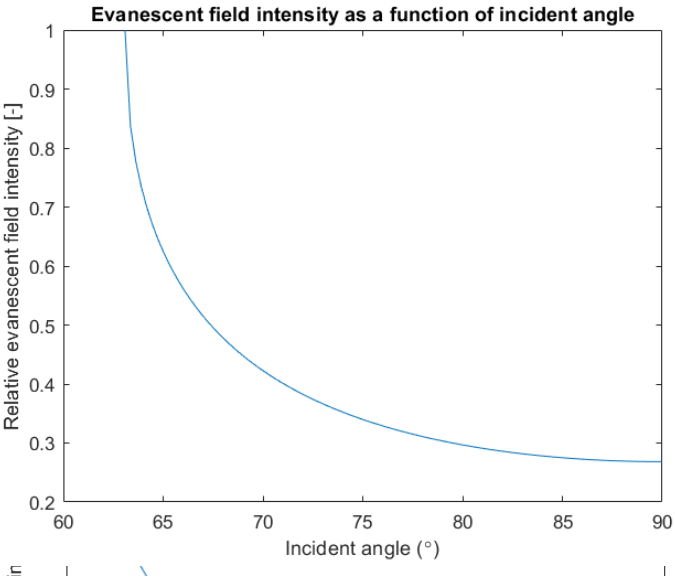

Figure 13: Evanescent field intensity as a function of incident angle

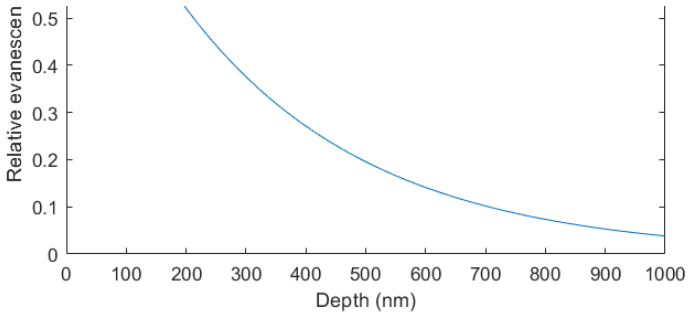

Figure 14: Evanescent field intensity as a function of depth

9.6 Calculations for estimation of MNP velocity and magnetic force

Upon magnetic actuation, the velocity of an MNP in a fluid depends on two forces: the magnetic force and the drag force, which are given by equations 4 and 5.

$$F_m = \frac{4}{3}\pi r^3 X \nabla\left(\frac{B^2}{2\mu_0}\right) \quad (4) \qquad F_d = 6\pi\eta r v_{bead} \quad (5)$$

In steady-state $F_m = F_d$ and the velocity of the particles can be estimated assuming that the field gradient is constant across the volume of the cartridge:

$$v_{bead} = \frac{1}{9} r^2 \frac{X}{\mu_0 \eta} \nabla B^2 \quad (6)$$

The magnetic flux density of the permanent magnets used in the magnetic actuation stage was measured using a Gaussmeter. The field gradient was computed and used to calculate the velocity of the bead.

Furthermore, the following constants were used:

- $r = 500 \cdot 10^{-9}\ m$
- $X = 81 \cdot 10^{-5}$
- $\mu_0 = 4\pi \cdot 10^{-7}\ N/A^2$
- $\eta = 1.13 \cdot 10^{-3} Ns/m^2$
- $\nabla B^2 = 127\ T^2/m$ at 5mm from magnet

Using a value of $\nabla B^2 = 127$ at 5 mm distance from the permanent magnet, the calculations yielded a velocity of $7.13 \cdot 10^{-4}$ m/s. As the flow cell sticker has a height of 0.8mm the time needed to pull MNPs from the sensor surface to the top of the flow cell sticker equals

$$t = \frac{8 \cdot 10^{-4}}{7.13 \cdot 10^{-4}} = 1.12\ s$$

By plugging in the velocity of the MNP in formula 5 the magnetic force was estimated to be 9 pN.

9.7 Multiplex Fluorophores

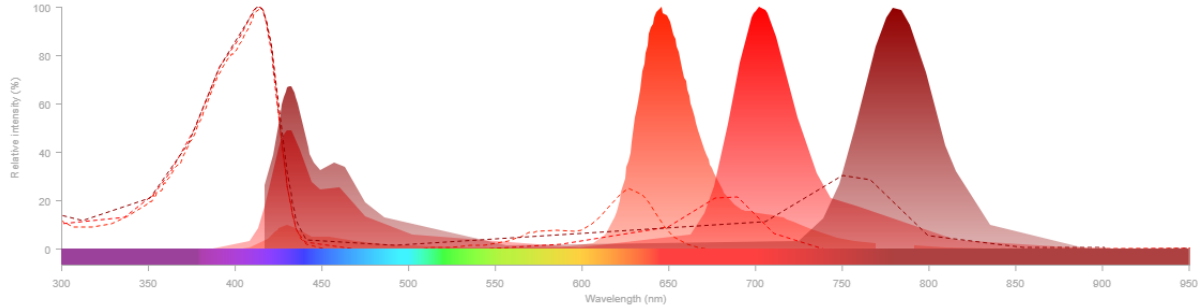

Figure 15: Excitation (dashed lines) and emission (filled area) peaks of Super Bright 645 (SB645), Super Bright 702 (SB702) and Super Bright 780 (SB780), from left to right respectively. [32]

9.8 Market share

The estimation for the size of the Dutch market for sepsis diagnosis is based on the prevalence and the size of the global market. Globally around 50 million people are affected by sepsis, leading to a diagnosis market of 615 million USD, being 15 % point-of-care tests. In Europe 3.4 million people are affected, hence the market size is calculated as 6 277 080 USD for point-of-care tests.

Table 1: Point-of-care sepsis diagnosis market growth

	CAGR	2021	2027	2028	2029	2030
Global sepsis diagnosis	9,6%	$ 615.400.000	$ 1.066.647.247	$ 1.169.045.383	$ 1.281.273.739	$ 1.404.276.018
Global POC sepsis diagnosis	5,0%	$ 92.310.000	$ 115.117.089	$ 119.146.187	$ 123.316.304	$ 127.632.374
Europe POC sepsis diagnosis	5,0%	$ 6.277.080	$ 7.827.962	$ 8.101.941	$ 8.385.509	$ 8.679.001

Using the compound annual growth rate (CAGR) method, the ending value of the European Market after 5 years is calculated as 430 500 USD. After 5 years, a CAGR of 5% for the European market results in a market value of 7,8 million USD in 2027.

9.9 Assessment of market potential, per customer segment

To assess the market potential of our biosensor, first an overview was made of all the customer segments. This was defined as the market opportunity set:

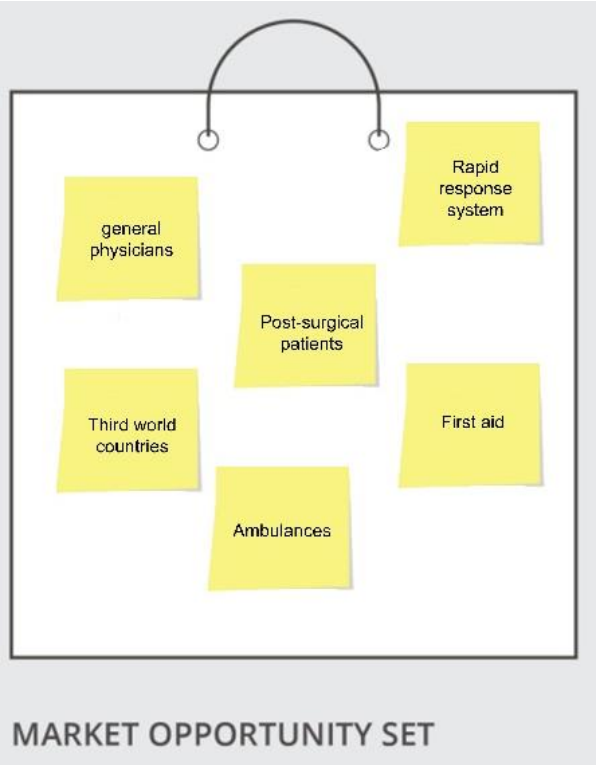

Figure 16: Market Opportunity set

Subsequently both the market potential and market challenges have been assessed for all the customer segments and put into the following tables:

- Market volume has been quantified on a scale of 1 – 5, with 1 is smallest and 5 is biggest market volume.
- Economic viability has been quantified on a scale of 1 – 5, with 1 is smallest and 5 is biggest economic viability.

Table 2: Evaluation of market potential per customer segment

Customer segment	Compelling reason to buy	Market volume	Economic viability
General Practitioners	Earlier diagnosis in the risk group can reduce the number of ICU-admissions by 6% (see calculation in appendix 9.3). In addition to that, the risk group will only grow in the upcoming years [18].	3	3
Ambulances	The unmet need is not very large as the test would mostly be used to be sure that this is a case of sepsis in which they most likely have been put on antibiotics. In trauma cases a biosensor for diagnosis has no additional value [6].	2	3
Post-operative patients	It depends on how early we can detect sepsis and how many tests have to be done. If there are many tests needed, it is a less effective solution. Large risks for false positives because IL-6 is already spiked after surgery [6][7].	2	3
RRS (rapid response system)	Less compelling as the diagnosis is often already made fast and antibiotics will already have been administered [7]	2	3
Hospitals in 3rd world countries	Additional reason because antibiotics can be used more effectively, and hence not really a solution for antibiotic shortages) [23].	5	1
First Aid (emergency room)	Solution can help for a part of all incoming patients, but not for trauma cases [6].	2	3

Table 3: Evaluation of market challenges per customer segment

Customer segment	Implementation difficulties	Time to revenue	External risks
General Practitioners	Distribution to GP offices is more difficult, compared to hospitals. GP offices are spread out throughout the country. In the future T.E.S.T. can collaborate with distribution and supplier companies, such as Certe [8].	Market would be ready for a solution for earlier diagnosis, since GPs are already contacting hospital doctors for advice on referring potential sepsis patients [7][8][9]	GPs might prefer still the available solutions such as CRP point-of-care testing, since these are longer on the market and accurate [24].
Ambulances	Ambulances are always on the move and paramedic are always in somewhat of a hurry. This means that our biosensor must be robust and work on the road while remaining reliable. Results should still be available as soon as possible, preferable within 5 minutes.	It will take more time to revenue, since the design of the biosensor needs to be adapted (to be robust, small, easy, and fast to use) compared to other costumer segments.	The paramedics need training in the usage of the biosensor which will cost a lot of training hours, whereas precautionary antibiotic treatment is still a very safe and lower cost option in developed countries.
Post-operative	Implementation of the biosensor should be straightforward. Nurses and the hospitals are familiar with biosensors and similar kinds of tests, for which they must draw blood. Next the sensor could be easily set-up in the clinical lab of the hospital.	It takes time to convince the market of the necessity and added value of T.E.S.T.'s biosensor compared to laboratory testing with a high accuracy.	There are a lot of other tests that are already part of hospital workflow which makes the addition of one more a difficult task [6][8][10].
RRS (rapid response system)	Implementation of the biosensor should be straightforward. Nurses and the hospitals are familiar with biosensors and similar kinds of tests, for which they have to draw blood. Next the sensor could be easily set-up in the clinical lab of the hospital.	It takes time to convince the market of the necessity of T.E.S.T.'s biosensor, and added value of T.E.S.T.'s biosensor, compared to the existing fast alternative in this customer segment (blind	Usage of diagnostic biosensor could become a problem since RRS have to act in a split second and having to wait on a biosensor to give results is no option in these cases.

		admission of a large range of antibiotics) [7].	
3rd world countries' hospitals	Hard to find funding since other problems such as hygiene are of bigger importance than a biosensor to prevent sepsis [23].	Market could be hesitant about buying the biosensor since funding is needed more in different healthcare solutions [23].	Correct shipping and training the clinicians could be potential risk factors [23].
First aid (emergency room)	Implementation of the biosensor should be straightforward. Nurses and the hospitals are familiar with biosensors and similar kinds of tests, for which they have to draw blood. Next the sensor could be easily set-up in the clinical lab of the hospital.	It takes time to convince the market of the necessity of T.E.S.T.'s biosensor, and added value of T.E.S.T.'s biosensor, compared to the existing fast alternative in this customer segment (blind admission of a large range of antibiotics) [7].	There are a lot of other tests that are already part of hospital workflow which makes the addition of one more a difficult task [6][8][10].

These tables resulted in the following attractiveness map and agile focus dartboard:

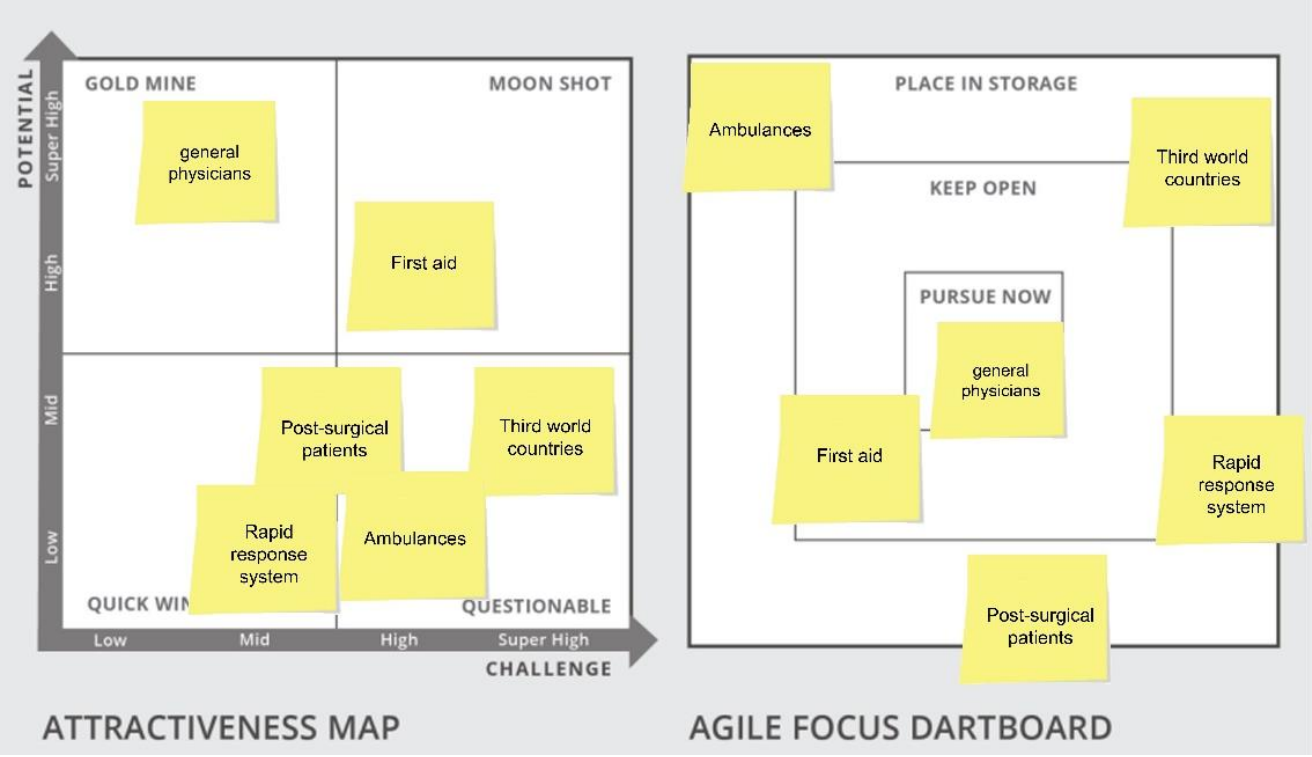

Figure 17: Attractiveness map and agile focus dartboard of possible sepsis point-of-care biosensor.

From all the research that has been done on all the different customer segments we concluded to focus on implementation of our biosensor in practices of GPs.

9.10 Money saved in the health care system in the Netherlands

The number of septic patients that are admitted to the ICU in the Netherlands is 10 000 annually, from which 48% had visited the GP and in 43% of those cases the GP failed to diagnose correctly. Hence 10 000 x 0,48 x 0,43 = 2 064 patients could be prevented for admission on the ICU by using T.E.S.T.'s biosensor in the GP consult annually.

2 064 / 35 000 = 6% of the total septic patients will be prevented to be admitted to the ICU annually.
2 064 patients x 1 300 EURO for ICU a day x 17 days = 45 614 400 EURO

The total gain for the healthcare system is expected to be higher at 50 000 000 EURO due to earlier diagnosis, less days on the ICU and less long-term care for septic patients.

9.11 Patient Journey

Persona: male of 86 years old, living still at home alone.
Scenario: male has a fiver of 39 degrees,
Goals: diagnosis sepsis as soon as possible

Table 4: Patient journey based on a real patient case

Awareness:	**Consideration:**	**Application:**	**Treatment:**	**Monitoring:**
An 86-year-old man arrives to the doctor's office with a fever and a body temperature of 39 degrees Celsius, but he has no idea how sick he is.	When the symptoms are generic, the doctor may suspect the beginning of an acute inflammation. She can start with multiple time-consuming tests or utilize the T.E.S.T.'s biosensor first to rule out acute inflammation or sepsis.	T.E.S.T.'s biosensor can be employed in this situation. The sensor will provide a result in 5-10 minutes, allowing it to be used immediately during the (consult) with the patient.	The doctor now knows what sort of antibiotics the man requires for at home, or whether he has to be taken to the hospital's intensive care unit right away.	Depending on the type of therapy, the GP can keep track of the patient's antibiotic medication at home. The hospital will also keep the GP informed about the recovery process. When the septic patient is discharged from the hospital, the GP will become more involved again, in the long-term therapy and recovering process.

9.12 Patient Journey

STRENGTHS
- T.E.S.T. consists of students that are supported by research groups at TU/e that have knowledge of the applied sensing principles. During the initial phase of product development continued support will be available consisting of laboratory usage and advice. The university is equipped to support start-ups by applying for patents and incorporate start-ups in the division TU/e participations including financial support. - In addition to the SensUs competition, T.E.S.T. made it to the final of the TU/e contest 2022. The TU/e contest gave the team a large platform to present their biosensor to new investors and companies, resulting in a lot of knowledge, advice and new partners for the business model of the sensor. Team TEST has built already a big network of partners and other stakeholders, alle enthusiastic about the development of the sensor. - Finally, T.E.S.T.'s biosensor has the potential to be converted into a multiplex biosensor that can assess many biomarkers simultaneously for diagnosing acute inflammations.
WEAKNESSES
- All team members are educated in Biomedical Engineering (BSc and MSc) at the same university. There are no students studying electrical engineering, industrial design, or technical business and entrepreneurship. - T.E.S.T. is relatively small and has limited resources (as compared to competing companies or start-ups). - Some components and approaches of the biosensor have already been (partially) patented, which makes the collaboration with partners and getting licensing more difficult. In addition to that, due to new regulations all notified bodies are flooded by many applications. - There is uncertainty about compensation by the insurance companies. This makes selling and pitching our biosensor to potential purchasers (GPs) and investors more challenging. - T.E.S.T. anticipates that persuading GPs to include our biosensor into their workflow and patient journey can be a bottleneck.
OPPORTUNITIES
- T.E.S.T. has presented their biosensor already on the TU/e contest which helped networking with partners and presenting themselves in the innovative technological field. In the future T.E.S.T. will continue participating and presenting themselves at other biomedical start-up markets and conferences, such as a Demo Day of Health Community or symposiums organized by Brainport Eindhoven. - The last pandemic demonstrated to the Netherlands the need for ICU bed availability. T.E.S.T.'s biosensor will hospital admissions and hence reduce the pressure on the limited available ICU beds. - As the population is aging the impact and occurrence of sepsis is also growing, so the market is growing slightly. - Some new patent rules will be introduced this year that make it easier to apply for all countries in the European Union at the same time saving cost. - When T.E.S.T. is fully operational, they will be able to contribute to one of the Sustainable Development Goals; to ensure healthy lives and promote well-being for all ages. These value propositions will help in the marketing and arise more awareness in the market.
THREATS
- More companies are focusing on IL-6 biosensors, such as the other student teams and start-ups developing biosensors as part of the SensUs competition. Therefore, the biosensor principle of T.E.S.T. must be unique and measure accurate and rapidly. - At this point, T.E.S.T. is still depending on the TU/e laboratory and supplies. As a result, they are vulnerable to high pricing and material shortage. A partnership with Future Diagnostics or OEMs is required. The manufacturing of parts can then be transferred to their well-equipped laboratories. This can also help to reduce the cost of the biosensor

9.13 Current costs of biosensor and cartridge materials

Table 5: Costs of prototype biosensor

Component	**Price**
Photodiode	€ 325
Notch filter	€ 295
Laser	€ 103
Raspberry pi	€ 69
Display	€ 60
Prism	€ 55
Power supply	€ 21
3D printed housing	€ 10
Various	€ 7
ADC	€ 5
Total	€ 950

Table 6: Costs of prototype cartridges

Component	**Price per unit**	**Needed per cartridges**	**Price per cartridge**
Glass cover slips	€35 per 100	1	€0,35
Flow cell stickers	€138 per 160	1	€0,86
Capture antibody	€383 per 1 mg	50 µL 500 nM	€0,15
Detection antibody	€383 per 1 mg	2 µL 13.3 µM per batch of max 665 cartridges	€0,00226
Dye	€342 per 1 mg	2 µL 1 mM per batch of max 200 cartridges	€0,00455
BSA	€79,90 per 10 g	60 µL 1%: 600 µg	€0,0048
MNPs	€602 per 2 ml	0.4 µl stock	€0,12
Total			€1,49

9.14 Number of expected tests and cartridges sold

The prediction for the number of sold tests in 2027 is based on a few assumptions based on (the only) data available [24]. T.E.S.T. anticipates that their sensor will be comparable to the CRP point-of-care sensor. According to statistics and studies from 2011, a GP office in the Netherlands found 936 CRP tests required, per 46 GP offices over a 2.5-month period. This study was conducted between January and March of 2011, and these CRP point-of-care tests are used for more than only identifying acute inflammation. The primary purpose of these tests was to limit the number of unnecessary antibiotic admissions. T.E.S.T. expects that this will remain the market's largest competitor since the products are so comparable. With T.E.S.T.'s future view to multiplex testing, the T.E.S.T. biosensor is expected to replace a portion of the CRP testing. T.E.S.T. will thus sell 100 tests per GP's office each year, compared to approximately 96 CRP tests in 2011 by the concurrent. This increased number is also based on the fact that the customer base (elderly >65) has expanded in the recent decade, as have the cases of acute inflammation [6][7][8][9][24].

936/46 = 20 CRP tests per 2,5 months, per GP's office
In one year, this gives 20 x 4,8 = 96 CRP tests per year, per GP's office
In the Netherlands 96 CRP tests per GP are performed on average per year as is calculated above. There are 454 000 GPs in Europe. Assuming CRP testing in the Netherlands is comparable to Europe as a whole, 45,4 million CRP tests are done annually. This is likely an overestimation of the number of POC CRP tests performed at the

GP as the Dutch healthcare system is quite advanced. To even that out the estimation of total tests in Europe is decreased to 40 million. For our market share this market is assumed to stay the same size. For each sensor sold the current and following years 100 tests are assumed to be performed. For each increase in market share sensors are bought.

Table 6: Market share in 2027-2030 based on POC CRP tests

	2027	**2028**	**2029**	**2030**
Share of tests for T.E.S.T.	0,0025	0,0075	0,015	0,025
Number of total tests	40 000 000	40 000 000	40 000 000	40 000 000
Tests performed	100 000	300 000	600 000	1 000 000
Additional sensors	1000	2000	3000	4000
CRP market value based on tests of €5	200 000 000	200 000 000	200 000 000	200 000 000

9.15 Overview of expected costs and revenue during 2023-2030

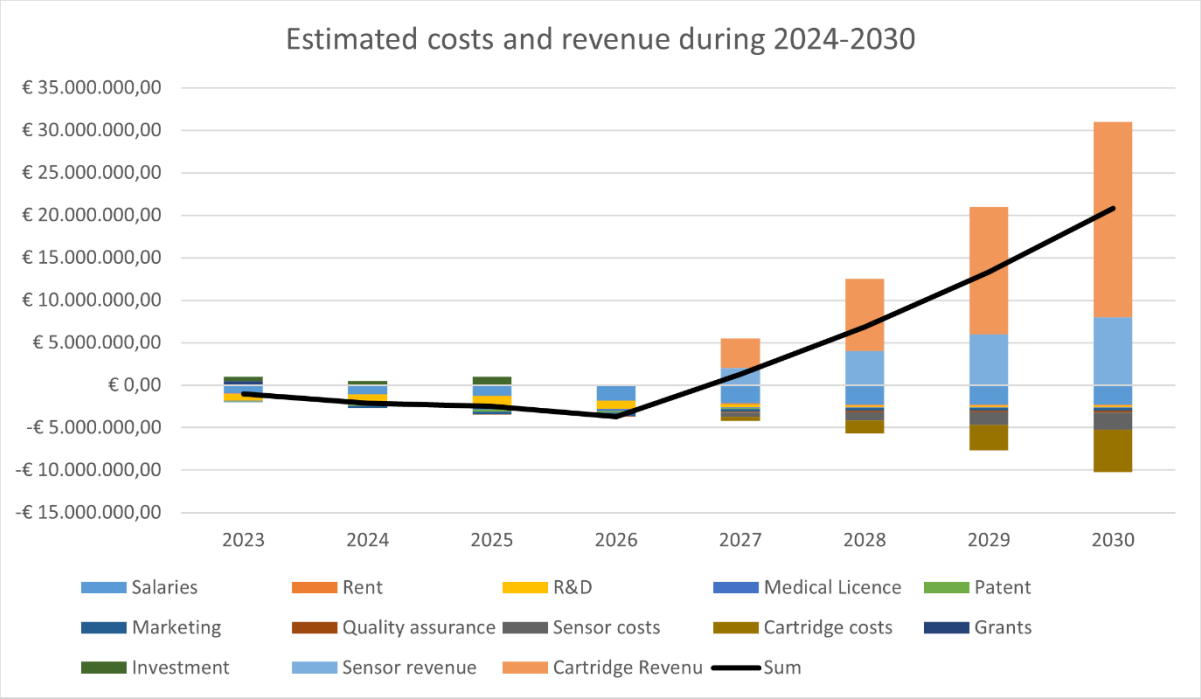

Figure 18: Estimated costs and revenue of T.E.S.T. biosensor from 2023 until 2030

In Figure 17 the costs, revenues and net gain can be seen. In the first year on the market there is already a profit of 1.2 million EUR. In table 7 the composition of costs can be examined further.

Table 7: Cost and revenue from 2023 until 2030 of T.E.S.T.'s biosensor

COST									
Phase	**R&D**		**NL pilot**		**EU market**				**TOTAL**
Year	**2023**	**2024**	**2025**	**2026**	**2027**	**2028**	**2029**	**2030**	
Nr. employees	*11*	*12*	*14*	*20*	*24*	*26*	*26*	*26*	
Salary per employee	€ 90.000	€ 90.000	€ 90.000	€ 90.000	€ 90.000	€ 90.000	€ 90.000	€ 90.000	
Salaries	€ 990.000	€ 1.080.000	€ 1.260.000	€ 1.800.000	€ 2.160.000	€ 2.340.000	€ 2.340.000	€ 2.340.000	€ 14.310.000
Rent	€ 35.000	€ 35.000	€ 35.000	€ 35.000	€ 70.000	€ 70.000	€ 70.000	€ 70.000	€ 420.000
R&D	€ 800.000	€ 1.000.000	€ 1.200.000	€ 1.000.000	€ 400.000	€ 200.000	€ 200.000	€ 200.000	€ 5.000.000
Medical Licence	€ 50.000	€ 200.000	€ 400.000	€ 200.000	€ 50.000	€ 50.000	€ 50.000	€ 50.000	€ 1.050.000
Patent	€ 100.000	€ 150.000	€ 200.000	€ 200.000	€ 100.000	€ 10.000	€ 10.000	€ 10.000	€ 780.000
Marketing	€ 50.000	€ 200.000	€ 300.000	€ 400.000	€ 300.000	€ 300.000	€ 300.000	€ 300.000	€ 2.150.000
Quality assurance	€ 0	€ 0	€ 100.000	€ 100.000	€ 150.000	€ 200.000	€ 230.000	€ 250.000	€ 1.030.000
Nr. sensors					*1000*	*2000*	*3000*	*4000*	
Production cost per sensor					*€ 500*	*€ 500*	*€ 500*	*€ 500*	
Production cost sensors					€ 500.000	€ 1.000.000	€ 1.500.000	€ 2.000.000	€ 5.000.000
Nr. cartridges					*100000*	*300000*	*600000*	*1000000*	
Production cost per cartridge					*€ 5*	*€ 5*	*€ 5*	*€ 5*	
Production cost cartridges					€ 500.000	€ 1.500.000	€ 3.000.000	€ 5.000.000	€ 10.000.000
TOTAL	€ 2.025.000	€ 2.665.000	€ 3.495.000	€ 3.735.000	€ 4.230.000	€ 5.670.000	€ 7.700.000	€ 10.220.000	**€ 39.740.000**
REVENUE									
Phase	**R&D**		**NL pilot**		**EU market**				**TOTAL**
Year	**2023**	**2024**	**2025**	**2026**	**2027**	**2028**	**2029**	**2030**	
Grants	€ 500.000								€ 500.000
Investment		€ 500.000	€ 1.000.000						€ 1.500.000
Nr. sensors					*1000*	*2000*	*3000*	*4000*	
Revenu per sensor	*€ 2.000*	*€ 2.000*	*€ 2.000*	*€ 2.000*	*€ 2.000*	*€ 2.000*	*€ 2.000*	*€ 2.000*	
Revenu sensors	€ 0	€ 0	€ 0	€ 0	€ 2.000.000	€ 4.000.000	€ 6.000.000	€ 8.000.000	€ 20.000.000
Nr. cartridges					*100000*	*300000*	*600000*	*1000000*	
Revenue per test	*€ 15*	*€ 15*	*€ 15*	*€ 15*	*€ 15*	*€ 15*	*€ 15*	*€ 15*	
Revenue cartridges	€ 0	€ 0	€ 0	€ 0	€ 1.500.000	€ 4.500.000	€ 9.000.000	€ 15.000.000	€ 30.000.000
TOTAL	€ 500.000	€ 500.000	€ 1.000.000	€ 0	€ 3.500.000	€ 8.500.000	€ 15.000.000	€ 23.000.000	**€ 52.000.000**