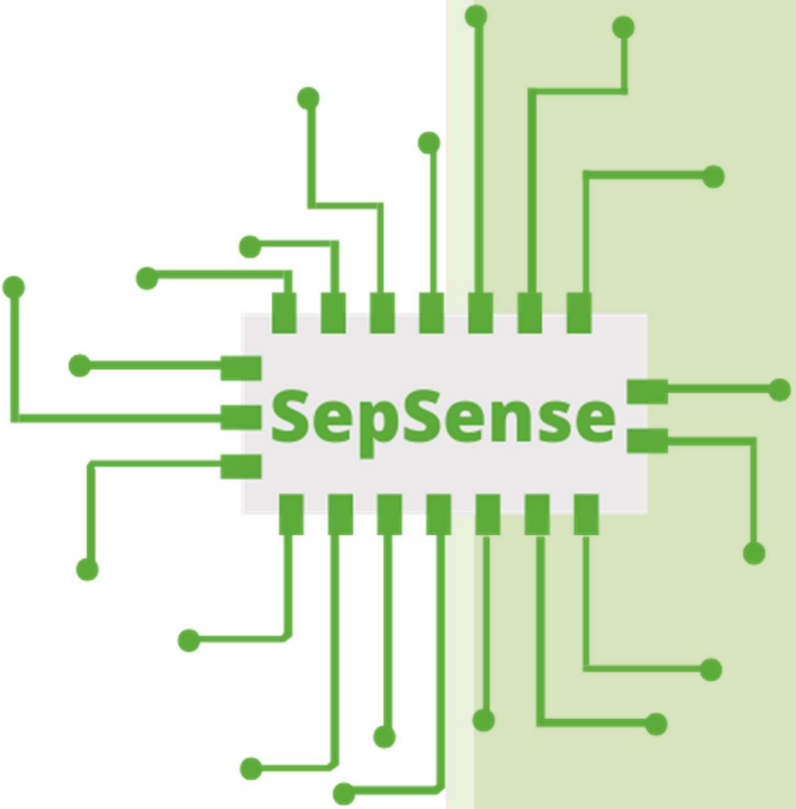
# SepSense - Team Results Document

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## 1. Abstract

*We developed a novel sandwich, self-calibrating, fully automated optical immunosensor to measure the concentration of IL-6 in human plasma as a biomarker for rapid sepsis detection.*

*Our biosensor design includes an optical system comprising of a camera and optical filter sets to measure the fluorescence from the immunoassay and the IL-6 concentration is calculated using signal processing techniques. Auto-calibration is uniquely integrated through second immuno-sandwich structure that involves a primary antibody attached to a glass surface which is then bound to a calibration fluorophore, itself linked to a secondary antibody, which serves as a bridge with the primary IL-6 detection sandwich, through a biotin-streptavidin link. The ratio between the measured intensity of the detection fluorophore and that of the calibration fluorophore allows us to quantify IL-6 concentration accurately by eliminating the variations arising from using different devices, by improving quality control internally.*

*A disposable microfluidic cartridge contains the detection surfaces and handles the sample preparation operations. This configuration of the biosensor allows fully automated, rapid, and precise measurement of the IL- 6 concentration and eliminates the hurdles of biosensors calibration requirements. Furthermore, this report includes SepSense's business proposal and future plans for the development and commercialization of the sensor. Involving funding, development, mass production and distribution of the biosensor locally and then globally.*

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## 2. Biosensor System & Assay

### 2.1. Double Immuno-sandwich Biosensor Structure & Reagents

This prototype incorporates a self-calibrating element based on the saturation of anti-BSA antibodies with Tetramethylrhodamine (TRITC) labelled bovine serum albumin (BSA) on a glass surface and combines the calibration and detection components in a double immuno-sandwich structure through a Streptavidin-Biotin complex to increase sensitivity (figure 1).

The surface-directed immobilisation of the primary antibody (anti-BSA antibody) is achieved using (3-dimethylaminopropyl)-3-ethylcarbodiimide(EDC)-N-hydroxysuccinimide (NHS) coupling due to its efficiency (Fischer, 2010). To achieve this, the glass is first sterilised by sonication using acetone and ethanol for 10 minutes. 2% of 3-aminopropyltriethoxysilane in methanol is then utilised to activate amines on the glass surface for an estimated 90 minutes. The amine-activated surface is reacted with 11-Mercapto undecanoic acid (MUA) and 3-Mercapto propionic acid (MPA) in a 1:9 ratio for 18 hours at room temperature. This step generates the carboxylic acid group, required for molecular binding on the glass substrate (appendix 9.2).

Next, the primary antibody is activated in 0.1M 2-(N-morpholino)ethanesulfonic acid (MES) and reacted with NHS-EDC on the activated glass surface which allows for the anti-BSA antibody to attach to the glass surface. Anti-BSA biotin is then allowed to bind to the labelled BSA after the surface is blocked with 1% casein in 0.01M Phosphate Buffer Saline (PBS). The biotin conjugate of this antibody allows it to bind to the Streptavidin-Biotin complex. To achieve this, the anti-IL6 antibody is dissolved in 10mM PBS, pH 7.2 and biotin succinimidyl ester solution (SE) is dissolved in the Dimethyl sulfoxide (DMSO) to obtain a 10mg/ml solution (Haugland and You, 2008). The biotin-SE is slowly added to the dissolved antibody and stirred. The mixture is incubated at room temperature for 1 hour with gentle agitation and purified using a gel filtration column. To avoid denaturation, the biotinylated antibodies are stabilised by adding BSA at a final concentration of 0.1%. The human plasma is then introduced and binds to the anti-IL6 biotin antibody. Finally, the detection antibody, anti-IL6 FITC, attaches to the available IL6 molecule in the patient plasma emitting green fluorescence which is then detected the optical detection system. To preserve the antibodies after detection, the glass slides are stored in PBS at 4°C. A washing step with a solution of PBS and 0.05% Tween-20 is performed following each stage of antibody attachment. All antibody and plasma binding occurs at room temperature for 1 hour.

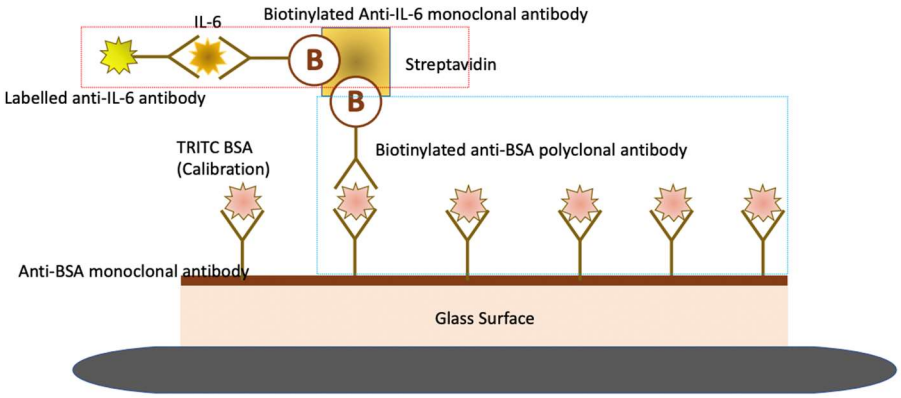


Figure 1: Schematic representation of the biochemistry proposed for the biosensor. The self-calibration part is denoted by blue and the detection by red.

### 2.2 Physical Transduction

As seen from figure 2, an optical system is utilised to quantify IL-6 concentration through fluorescent measurement due to the high sensitivity of the optical system and its cost effectiveness. The system comprises of a camera sensor with the appropriate excitation\emission filter set to measure the fluorescence of the labelled IL-6 capturing antibodies. Firstly, a self-calibration stage is initiated by the microcontroller, in which the calibration fluorophore is excited with a bright light source (20,000 mcd) using the corresponding excitation filter (540 nm bandpass). Following this, the camera captures an image for the sensing region through the corresponding emission filter (590 nm bandpass filter) and subsequently image processing techniques are applied to measure the intensity of the calibration fluorophore and record the value. Next, the second stage of IL-6 detection is performed to measure the intensity of the detection fluorophore in a similar way to that of the calibration fluorophore but with different excitation\emission filters (470 nm - 525 nm respectively). Finally, the ratio between the two measured intensities is calculated internally in the microcontroller and correlated to a standard curve to provide the corresponding IL-6 concentration.

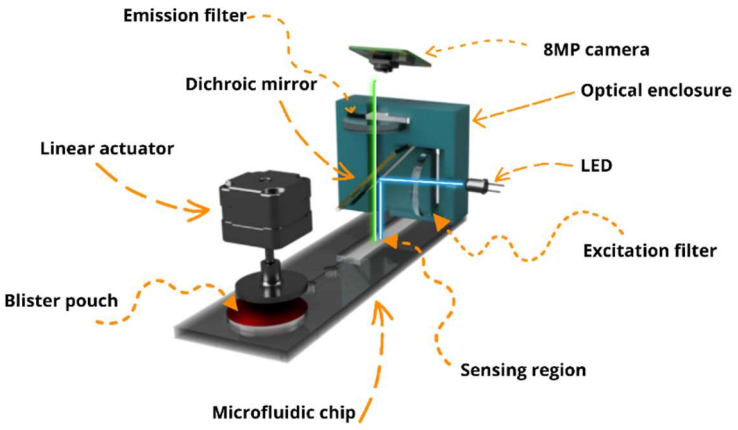


Figure 2. General design of the biosensor indicating different parts of the system

## 2.3 Cartridge Technology

The disposable cartridge design was manufactured from polymethyl methacrylate (PMMA) sheets with a 2 mm thickness and its dimensions are presented in Figure 1.

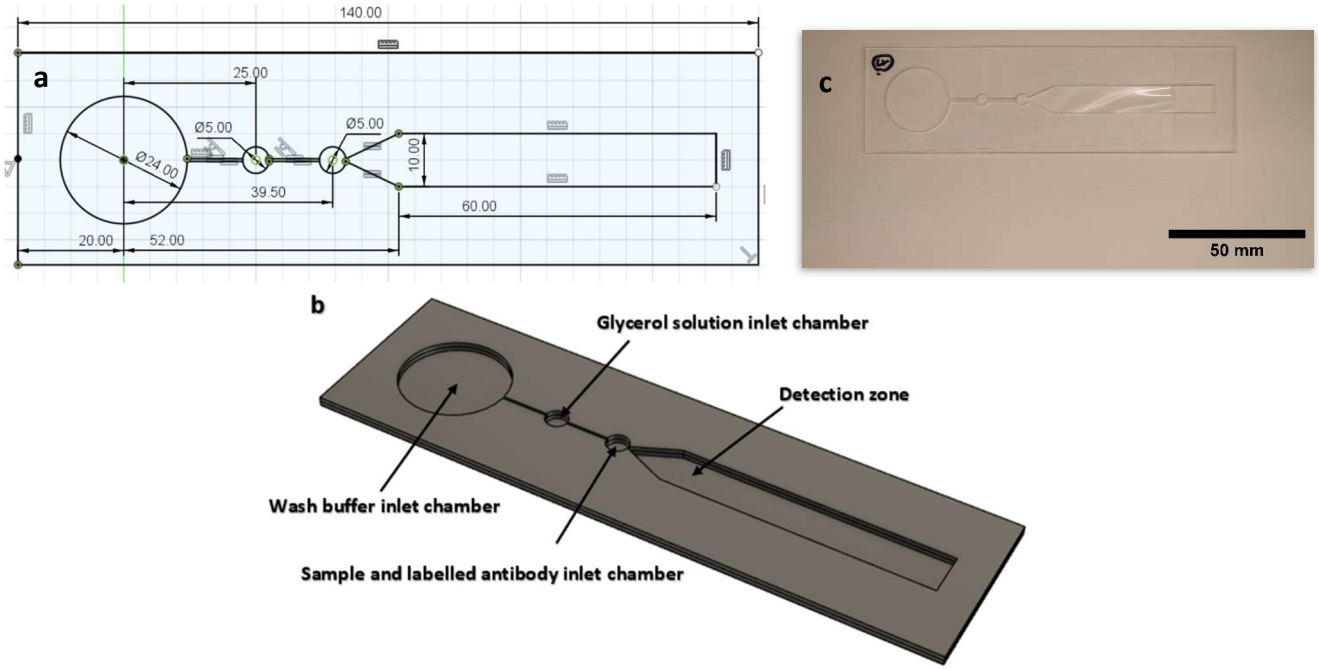


Figure 3: a) 2D schematic and dimensions un mm of the proposed cartridge and b) 3D schematic of the proposed cartridge laser cut with a thickness of 2 mm c) laser cut design of the proposed cartridge (scalebar 50 mm).

As seen in Figure 3, there are a total of three inlets in the cartridge leading into the detection zone. The first inlet is for the addition of the wash buffer, the second for the glycerol water solution (50 % by weight) to reduce the diffusion between the sample and wash buffer and to control their flow (McNeely, 1999) and the third for the addition of the sample and the secondary labelled antibody. As PMMA is hydrophobic (Huang et al., 2010) and the plasma sample and other required reagents which have similar properties to that of water, to allow for flow movement and control the flowrate and thus allowing the unidirectional flow and mixing of that of the reagents, a pressure-based system which is still under development is proposed using a step motor which will press on the wash buffer chamber and thus allow for flow movement and control.

For the system to function, first the top layer and part of the bottom layer of the cartridge is covered in an adhesive film with holes to insert the solutions and sample and the bottom layer starting from the detection zone with the treated glass. 900 µl of the wash buffer is then added to the wash buffer inlet chamber, followed by 20 µl of the glycerol-water mixture and finally 20 µl of sample and the secondary labelled antibody. The holes in the top layer are then covered with an additional layer of adhesive film and the step motor utilised to press on the filled wash buffer chamber for fluid flow and for the sample and antibodies to come in contact with the treated surface in the detection zone and bind to it for the detection process. The wash buffer removes any additional unbound antibodies and waste, however, is slowed down by the glycerol-water mixture to allow for the complete binding of the antibodies to the surface. The entire process has an estimated duration of 60 seconds which is controlled by the speed and thus pressure exerted by the step motor.

# 3. Technological Feasibility

## 3.1 Detection System

Initially, the detection system was tested with different concentrations of pure fluorophore to find the limit of detection of the biosensor and ensure that the system design is working as desired. Different configurations were tested to achieve the maximum signal-to-interference ratio allowing for epifluorescence configuration to be selected as it resulted in the highest sensitivity in comparison with other methods. The system configuration is presented in the appendix 9.4.
The limit of detection was estimated to be approximately 30 pg/mL, as shown in Figure 4, which allows for the detection of the IL-6 concentration ranges of classes II to V of sepsis which will be tested during the Eindhoven event.

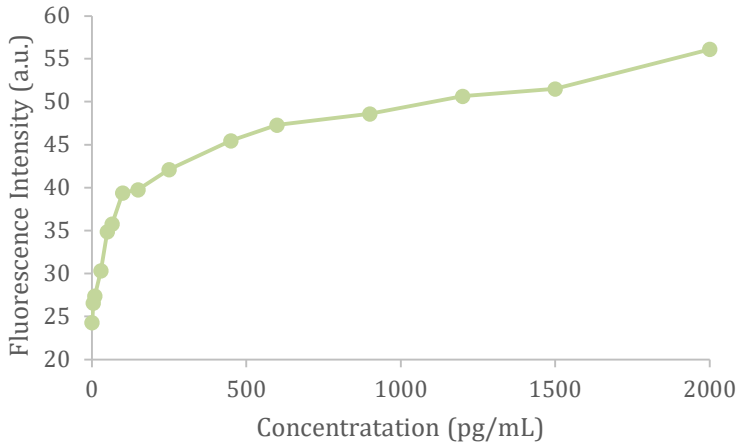


Figure 4. The fluorescence intensity measured over different concentrations of Alexa Fluor 488.

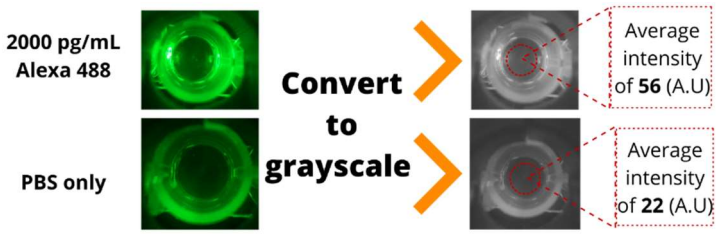


Figure 5. Raw images from the camera sensor and intensity extraction using image processing techniques.

## 3.2 Autocalibration

As calibration is a complex procedure requiring various resources, an autocalibration feature was implemented in the design to provide increased reliability and simplify the analysis procedure. To achieve this, two intensity measurements are taken from each sample, the first for the calibration fluorophore which has a known concentration, and the other measurement for the detection fluorophore which is bound to IL-6 molecules. By calculating the ratio between these two measurements for each sample, the system is able to provide accurate and reliable results despite the variations that are caused during assay functionalisation. The results of the validation experiment for the proof the concept of the autocalibration is presented in table 1.

Table 1. fluorescence intensity readings for various samples

| Sample number | Streptavidin concentration (Alexa Fluor 488) | BSA concentration (TRITC) | Streptavidin intensity (Alexa Fluor 488) | BSA intensity (TRITC) | Ratio |
|---|---|---|---|---|---|
| **1** | 1000 pg/mL | 5 ug/mL | 66.5 | 26.6 | 2.5 |
| **2** | 1000 pg/mL | 5 ug/mL | 52.7 | 20.2 | 2.6 |
| **3** | 500 pg/mL | 10 ug/mL | 47.9 | 29.9 | 1.6 |
| **4** | 1500 pg/mL | 10 ug/mL | 63.7 | 21.6 | 2.9 |

The first two samples were prepared in an identical manner resulting in equal concentrations to demonstrate the differences arising due to various factors, such as binding efficiency and geometry variation. From figure 6, the variations in florescent intensity between samples 1 and 2 which have identical concentrations can be clearly observed. However, the autocalibration ratio for these two samples remains approximately similar, confirming that this method can be utilised to provide accurate measurements to estimate the true concentration of IL-6 in the human plasma samples, despite manufacturing inconsistencies. On the other hand, samples 3 and 4 have different concentration of the detection fluorophore which resulted in different intensity ratios (as higher ratios correspond to higher concentrations).

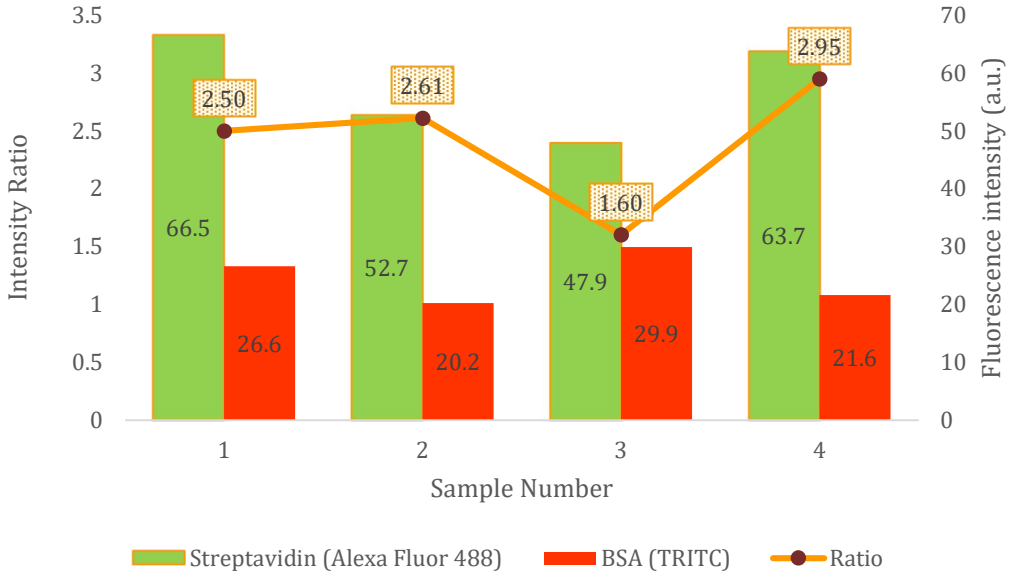


Figure 6. Ratiometric fluorescence plots for the visualisation of the concept of the autocalibration

## 3.3 Cartridge Technology

As the basis of the design is an optical biosensor, requiring a transparent apparatus for the detection of fluorescent signals, polymethyl methacrylate (PMMA) was selected for the manufacture of the proposed disposable cartridge for the unilateral fluidic delivery system of the sample and secondary labelled antibodies. This was due to its cost-effectiveness, optical transparency and biocompatibility, all of which are essential whilst designing a reusable optical biosensor (Trinh et al., 2020). Additionally, as IL-6 has a hydrophilic outer layer (Kalai et al., 1997), it could potentially adhere to hydrophilic surfaces and as such lower the detection rate of the biosensor, therefore the use of a hydrophilic material, such as PMMA (Toh et al., 2008), was paramount for the fluidic delivery system to counter this effect.

Although most biosensors of this scale rely on microfluidics for sample and reagent delivery to the detection zone, millifluidics and laser-cutting were utilised in this design as the flow is still highly controllable and in the laminar region required for the optical biosensors such as this with dimensions in the millimetre range in addition to being far simpler to manufacture and requiring less complicated fabrication methods such as lithography (Chen et al., 2021).

To verify and finalise the design, the mixing and diffusion of the fluids and samples in the detection zone are currently being investigated by measuring the colour change in that area. This is achieved by calculating the RGB values and mixing index of that region (Mahmud et al., 2020) utilising water-based dyed solutions of various colours representing the wash buffer (blue), glycerol-water solution (red) and the sample and labelled antibodies (green) and by comparing it to that of the unmixed and fully mixed solutions.

# 4 Originality

## 4.1 Originality Statement – Team Captain

When conducting preliminary research at the beginning of our biosensor development process, we noted that a larger percentage of available research papers described non-optical biosensors, more specifically electrochemical sensors. The smaller percentage of optical sensors described in literature, while often faster than their electrochemical equivalents, often did not provide clinically relevant results. However, we were very impressed by the other advantages (speed, costs, etc...) of optical methodologies, so as a team, we set out to develop a novel, optical sensor that can be used at point-of-care and provide the results faster than and at a similar or better sensitivity and specificity as alternatives. One of the considerations, that also arose from discussions with partners, was linked to the challenges in quality control for such sensors. We wanted to ensure our sensor was as reliable as possible and thus decided to incorporate self-calibration in its design, as an attempt to eliminate performance variation. Calibration is often performed externally (through a calibration curve for example), which can be challenging for a product.

The novel features that make our biosensor stand out with respect to the scientific state of art, as well as the technology used to bring these novel ideas to life were brought up entirely by the team. These ideas were formed and recognized, by the team, as ideas with high potential during our early meetings with our supervisor. Our supervisor helped us adjust and refine our ideas to make sure they were feasible, by asking questions in areas he identified as potentially problematic. Then after further research the team would collectively decide if there was reason for concern and proceed accordingly. In some instances when obstacles were encountered in the more conventional aspects of our biosensor such as our microfluidics design, PhD students sharing our labs would offer their opinions.

## 4.2 Originality Statement – Supervisor

I can confirm that the team has develop their idea completely independently.
Some of the team members took a course on Biosensors as part of their curriculum, that I teach in. The course covers the basics of biosensing and provides examples of different sensor systems and strategies. Members of the team were thus exposed to optical sensors and sandwich assays, along with concepts of microfluidics. However, I should stress that they started their work from scratch, through literature searches of possible strategies.

They realised that optical sensing is often easier/faster to implement, which guided their choices. They also realised the significant challenge around quantification and quality control and early on raised the idea of integrating a new internal calibration strategy. This is not a scheme that I had encountered before or that the wider group had researched.

I advised on potential challenges with the range of ideas they went through in their iterative process, but the solutions are wholly from their reflections.

The rest of the approach (fluidics, detection circuits, signal analysis, etc…), although coming from robust engineering design thinking, is conventional. But here again, I must stress that the Team was not directly supported by our researchers. Although we have extensive experience in the area, there is no active on-going research at the moment in our group on these techniques. All in all, I am very proud of how the team focused on a difficult challenge and provided a workable solution.

*Dr Julien Reboud – Supervisor*

*Georgia Charalampous – Team Captain*

# 5. Translational Potential

## 5.1 Business Model Canvas

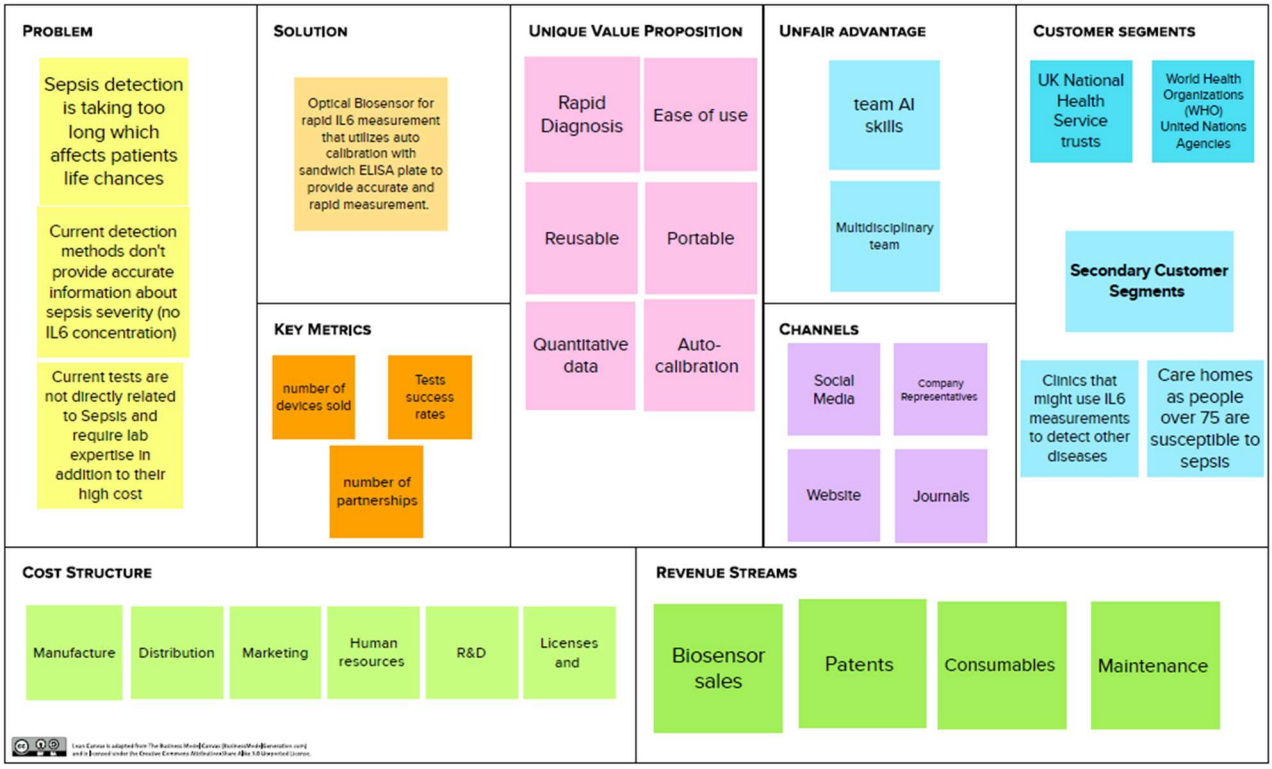


Figure 7: Business Model Canvas

## 5.2 Market Description & Stakeholder Desirability

Sepsis was declared a threat of global health by the World Health Organisation in 2017. In that same year, an estimated 49 million cases of sepsis and 11 million sepsis-related deaths occurred worldwide resulting in sepsis causing approximately 20% of all global deaths in 2017 (van den Berg, M et al 2022). Sepsis' burden on society extends beyond patient mortality as the quality of life of patients recovering from sepsis is often decreased post-hospitalisation and discharge. Around 50% of global sepsis survivors experience complications post treatment, with approximately one-sixth of sepsis survivors experiencing significant morbidity, such as functional limitations, moderate-to-severe cognitive impairment, and/or increased mental health disorders (World Health Organization, 2020. According to the National Confidential Enquiry into Patient Outcome and Death (NCEPOD) one in five patients in their UK study had evidence of complications when discharged (Alleway, R., 2022). These complications are presented in detail in the Appendix 9.2.

The management of the aforementioned complications, in addition to the costs relating to the initial treatment of sepsis during ICU and non-ICU hospitalisation contribute to the direct overall cost of sepsis on the National Health Service of the UK (NHS). Additional costs that should be considered when describing the full economical effect of sepsis on British society, include indirect costs relating to lost productivity and litigation as a result of sepsis. In total, the estimated annual direct and indirect cost of sepsis in the UK is between £7.42 billion and £10.2 billion. A potential reduction of 10% in hospital admissions due to sepsis could possibly result in a reported £83 million saving for the NHS annually. ( Allcatsrgrey.org.uk. 2022).

Literature reviews and interviews conducted with UK based clinicians and University of Glasgow Sepsis researchers have helped us define the current care pathway and patient journey as follows: patients exhibiting symptoms indicating sepsis are first assessed by a senior clinical decision maker through various tests to determine their sepsis risk score. The clinician also measures the patient's vital signs and takes their clinical history. Then depending on the results of the assessment, the patient is prescribed blood tests, such as blood culture and full blood count tests, and administered broad spectrum antibiotics until the specific cause of infection is identified (Nice.org.uk. 2022). These blood culture tests can potentially take up to 72 hours to confirm sepsis and identify the organism responsible for the infection. (Tsounidi, D. et al, 2021)

The Global Biosensors Market is estimated to be USD 28.12 billion in 2022 and is projected to reach USD 49.76 billion by 2027, growing at a Compound Annual Growth Rate (CAGR) of 12.09%. The invasiveness and time-consuming nature of traditional laboratory procedures, which are currently the gold standards for the diagnosis of several diseases including Sepsis, as well as the demand for disposable, cost effective and easy to use diagnostics are key factors contributing to the rapid growth of the biosensor market (Hassanalieragh, M. etl al, 2015, Shahini, A. 2016). Unlike the gold standard laboratory procedures,

biosensors can provide fast and precise disease diagnosis using small sample volumes at the point-of-care (PoC). The use of PoC biosensors also eliminates the need for elaborate sample treatment, expensive laboratory equipment and trained personnel which are required by traditional laboratory procedures (Tsounidi, D. et al, 2021).

Utilisation of biosensors for sepsis diagnosis not only has the potential to aid in the reduction of treatment costs and relieve the burden on the NHS, but most importantly, it will save lives, as early diagnosis is crucial for patient survival; each hour of delayed treatment is associated with an 8% rise in mortality (Tsounidi, D. et al, 2021). Due to this, our proposed fast, self-calibrating, easy-to-use, PoC optical biosensor can step in and disrupt care pathway by expediting diagnosis and decreasing the associated costs of sepsis for the already underfunded NHS system and improve survival rates across the UK. SepSense's user-friendly optical biosensor requires minimal handling by the clinician performing the test and can provide much faster results than a lab-based blood test.

## 5.3 Business Feasibility

Future actions for SepSense include further research to troubleshoot problems our prototype is currently facing in addition to optimising the sensor's functional features in an attempt to increase sensor marketability which requires an estimated 2 years.

While the R&D team continues the development in the background, the business team intends to attend more sepsis and point-of-care biosensor focused conferences as well as interact with more stakeholders, such as local sepsis patient groups (Sepsis Trust UK), representatives from local NHS Trusts and care homes. This is to expand our understanding of what the stakeholders need in a sepsis biosensor and what the process of new device adoption by NHS Trusts entails. These conversations with the NHS Trust representatives count as the first step in growing and developing a long-term customer connection.

The business team will also look towards joining the MedTech SuperConnector or similar accelerators to gain much the needed support to build this business through a network of professionals with experience in business development and finance which the team currently does not employ. Programmes, such as the MedTech SuperConnector, support early career researchers through funding, training in business development and access to industry networks all of which are required for a start up in its early stages of growth.

Finally, the business development team will strive to establish a partnership with HyTest to ensure reliable supply of the anti-IL-6 antibodies currently used in our assay. We will also look to partner up with a company with experience in developing medical devices in the UK that can mass manufacture our device and potentially assist in the obtainment of required certifications such as UKCA and CE marking for device commercialization in the UK.

## 5.4 Financial Viability

The global sepsis diagnostics market was valued at 596.6 million USD in 2021, registering a compound annual growth rate of approximately 8% during the period forecasted, that being from 2021 to 2027. Given the UK owns 2% of the global biosensors market, an estimated sepsis diagnostics market value of 120 million USD can be expected locally (Mordorintelligence.com. 2022) which corresponds to 245,000 sepsis patients annually in the UK, 48,000 of whom die from the disease. The large number of patients sets the NHS back approximately 2 billion pounds owing to treatment and diagnosis costs, where each treatment costs 20,000 pounds (Sepsis Trust. 2022). The current UK biosensor market size and great sepsis patient numbers, makes the UK an ideal starting point for the development and commercialisation of a novel sepsis biosensor. This belief is reinforced by the presence of the readily available infrastructure for research and development which accommodates the introduction of start up to fill this gap.

The design of our biosensor allows for the potential expansion into other major biosensor markets such as the US, where the country witnesses more than 970,000 sepsis cases annually, this disease accounts for more than 50% of deaths in hospitals and its treatment and the later stages causes an increased financial burden (Paoli, C. et al 2018).

The ultimate goal once the biosensor is fully developed and cost effective, is to expand to under-developed countries where the cases of sepsis are the highest (van den Berg et al 2022). Although a large consumer demand for this product exists in these countries, the limited infrastructure for biosensor mass production and distribution remains an obstacle. However, expected improvements in efficient and cost-effective methods of manufacturing may allow the deployment of our biosensor globally.

With a strong conceptual foundation for our biosensor, we are confident of its potential in making it to the commercial market. To achieve that goal, we aim to start off with a pre-seed start-up funding round, where the aim is to raise £ 75,000 from small ticket angel investors, with the aim to reach a final, workable, and fully automated prototype. Following that, we will pursue

seed funding to raise £ 250,000 from seed venture capitalists, with which the founding team will be supported by experienced members to take on vital roles ranging from product design, manufacturing, distribution, and advertising. With that we will be able to begin production of the prototype and provide the NHS with prototypes to test, aiming to demonstrate a more practical, efficient and affordable approach to sepsis screening. Throughout the trial stage we expect these NHS trusts to see the potential of our device in tackling sepsis. By the end of this period, we expect to start selling the biosensor first to the participating NHS trusts following the successful completion of the trial stage and then to other trusts made aware of our device. Then we will proceed with series A funding with the goal of raising £ 1.8m, from large ticket investors and venture capitalists, to scale up the production of both the biosensor and its disposable cartridge. The expansion of our company after series A funding is secured, will include employee recruitment. Further series funding rounds will be resorted to when it comes to realizing the subsequent goals of expanding to new markets such as that of the USA or developing countries where sepsis is prevalent.

Our expected cost for the manufacturing of the biosensor and its cartridge is outlined in the table 2 below.

Table 2: Device cost breakdown

| *Component* | *Cost (pounds)* |
|---|---|
| **Camera/Photodiode/Arduino/wires batteries light source etc** | 100 |
| **Filter (excitation and emission)** | 600 |
| **other components (microfluidics)** | 100 |
| **Cartridge (expected price)** | 1 |
| Expected cost | 801 |
| Selling price | 1647 |

Given the market size in the UK and the potential for introduction of a new biosensor, it is safe to set our target at 10% of the final sepsis biosensor market in the UK by the year 2033. Keeping in mind that the introduction of new biosensors is expected to extend for around 10 years, we have put together a table summarizing the financials predicted for this period. It shows a secure and realistic outlook even with the conservative metrics and projections considered.Table 3: Predicted Financials for next 10 years

| Market share (million $) | Year | Market percentage goal (%) | Revenue (Million $) | Funding ($) | Total revenue ($) | Expenditure ($) | Number of devices sold per year | Number of cartridges sold per year |
|---|---|---|---|---|---|---|---|---|
| **139.968** | 2022 | 0.0 | 0.0 | 95000 | 95000 | 46000 | 0 | 0 |
| **151.16544** | 2023 | 0.0 | 0.0 | 0 | 0 | 46000 | 0 | 0 |
| **163.2586752** | 2024 | 0.5 | 0.7 | 303000 | 1002840 | 500000 | 5 | 52501 |
| **176.3193692** | 2025 | 0.8 | 1.3 | 0 | 1322395 | 1000000 | 10 | 99204 |
| **190.4249188** | 2026 | 1.0 | 1.9 | 2185000 | 4089249 | 4000000 | 14 | 142854 |
| **205.6589123** | 2027 | 3.0 | 6.2 | 0 | 6169767 | 7000000 | 44 | 462848 |
| **222.1116252** | 2028 | 5.0 | 11.1 | 0 | 11105581 | 8000000 | 80 | 833127 |
| **239.8805553** | 2029 | 6.0 | 14.4 | 0 | 14392833 | 10000000 | 103 | 1079732 |
| **259.0709997** | 2030 | 7.0 | 18.1 | 0 | 18134970 | 12000000 | 130 | 1360463 |
| **279.7966796** | 2031 | 8.0 | 22.4 | 0 | 22383734 | 11000000 | 161 | 1679200 |
| **302.180414** | 2032 | 9.0 | 27.2 | 0 | 27196237 | 10000000 | 195 | 2040228 |
| **326.3548471** | 2033 | 10.0 | 32.6 | 0 | 32635485 | 8000000 | 235 | 2448273 |

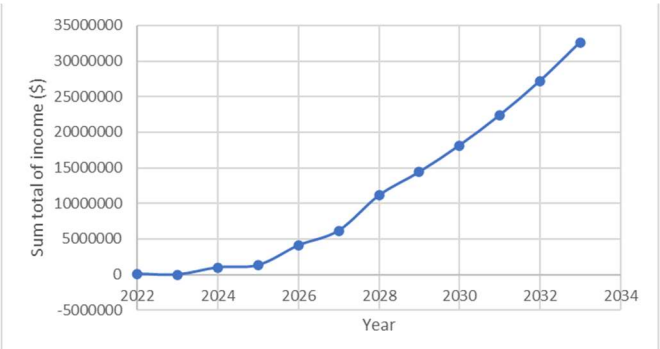


Figure 8: Income Projection to Market Share Goal

# 6 Team & Support

*6.1 Team Member Contributions*

Our team consisted of a number of sub-teams, consisting of Business (B), Biological Assay (BA) and Detection (D) teams:

| | |
|---|---|
| Abdullah AlFakhrey | Member of D and B, mainly responsible for the design of the optical system |
| Georgia Charalampous | Team captain and member of B mainly responsible for communication with SensUs organization and coordination of partner meetings. |
| Jaswinder Singh Hunjan | Member of D, mainly responsible for the design of the optical system |
| Maryam Nina Hardy | Member of BA, mainly responsible for antibody attachment to glass and assay development |
| Pilar Maria Ruiz Illescas | Member of BA, mainly responsible for assay development |
| Sara Shadman | Fluidic delivery system designer and responsible for the validation of the delivery system |
| Yazan Haidar | Member of D, BA and B, mainly responsible for preparation of autocalibration data. |

6.2 *External Support*

Over the previous months we have received support from the following people and organisations:

| | |
|---|---|
| Dr Julien Reboud | Supervisor and coach of the team, overlooking biosensor development and reader at the University of Glasgow |
| Andrew Phillips | University of Glasgow technician helped in getting access to electronics workshop and suggested use of components for the detection circuit |
| Dr Andre Glidle | University of Glasgow researcher giving advice on optical system design |
| Olivia McGleish | Assisted in the development of the MATLAB code for RGB data analysis |
| Medtronic | Have helped us troubleshoot problems with our electronics and optical system. |
| Sioux Technologies | Have helped us troubleshoot problems with our electronics and optical system. |
| Siemens Healthineers | Advised us on assay design business development |
| Handprints e-NABLE Scotland | University of Glasgow society aided in the printing of the 3D model |

## 7. Final Remarks

We would like to conclude this document by emphasizing how proud we are of our concept and what we have achieved in such a short period of time, despite not having access to labs early in our biosensor development process and being one of the last teams to join the competition. If given the chance, we would love to continue working on our biosensor after the completion of the competition to fully realise our concept and have our hard work payoff.

Furthermore, we would like to thank everyone who has helped us over the summer including but not limited to the following. First, we would like to thank our supervisor for giving us the opportunity to put our knowledge to the test on something the team had little prior experience in. Next, we would like to give thanks to the SensUs organisers, Anne-Lieke Craenen and Hans Douben in particular for, quickly responding to our questions despite how inconsequential some of them were in hindsight. Finally, we would like to thank the SensUs partner representatives for dedicating some of their precious time to help us along on our biosensor development journey.

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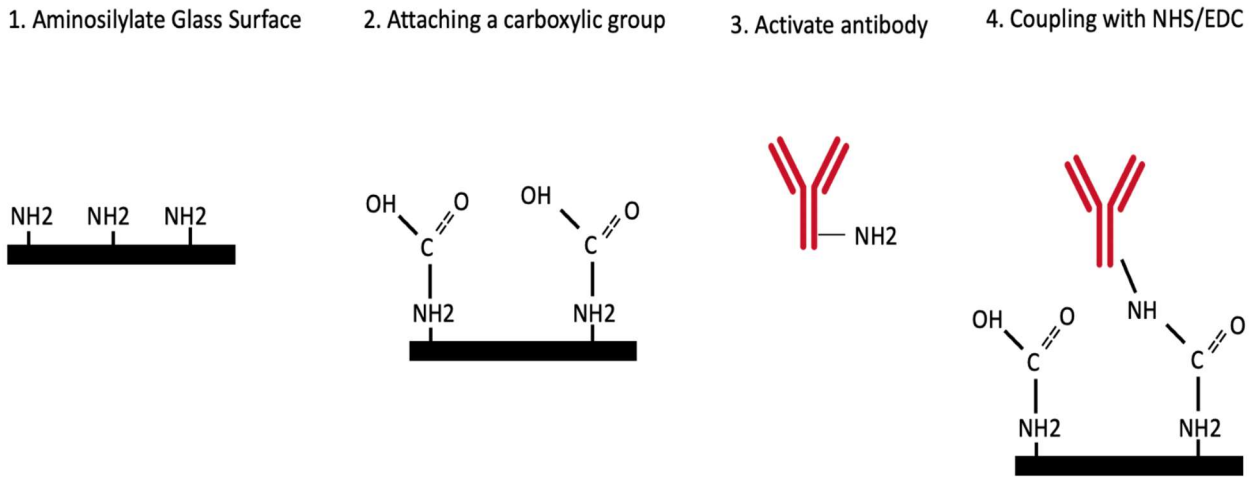
# 9. Appendix




Appendix 9. 1. Schematic diagram illustrating the binding of anti-BSA antibody to glass surface. Amines and carboxylic groups are attached to the glass surface and bound to the activated antibody using NHS-EDC coupling.

Appendix 9. 2: Rates of complications in ICU patients post-sepsis (Alleway, R., 2022)

| Complication | Rate (%) |
|---|---|
| Worsened physical function | 53.5 |
| Worsened cognitive state | 19.7 |
| Impaired kidney function | 14.1 |
| Post-sepsis syndrome | 5.6 |
| Wound problems | 5.6 |
| Chronic pain | 12.7 |
| Amputation | 8.5 |
| Tracheostomy | 4.2 |
| Post-traumatic stress disorder (PTSD) | 9.9 |
| Atrial fibrillation | 4.2 |
| Sepsis recurrence | 2.8 |
| Weight loss | 2.8 |
| Depression | 2.8 |
| Muscle weakness | 2.8 |

Appendix 9. 3: Summary of estimated costs of Sepsis on UK society and NHS (Alleway, R., 2022)

| | | Base case | Low estimate | High estimate |
|---|---|---|---|---|
| **Direct costs** | Hospital costs | £819,577,538 | £619,934,130 | £1,114,746,168 |
| | Complications | £9,180,688 | £1,836,138 | £18,361,376 |
| | **Total** | **£828,758,227** | **£621,770,267** | **£1,133,107,544** |
| **Indirect costs** | Productivity | £6,770,841,600 | £6,770,841,600 | £8,751,080,366 |
| | Litigation | £160,274,100 | £32,054,830 | £320,548,200 |
| | **Total** | **£6,931,115,700** | **£6,802,896,430** | **£9,071,628,566** |
| **GRAND TOTAL** | | **£7,759,873,926** | **£7,424,666,697** | **£10,204,736,110** |


Appendix 9. 4. Optical system configuration