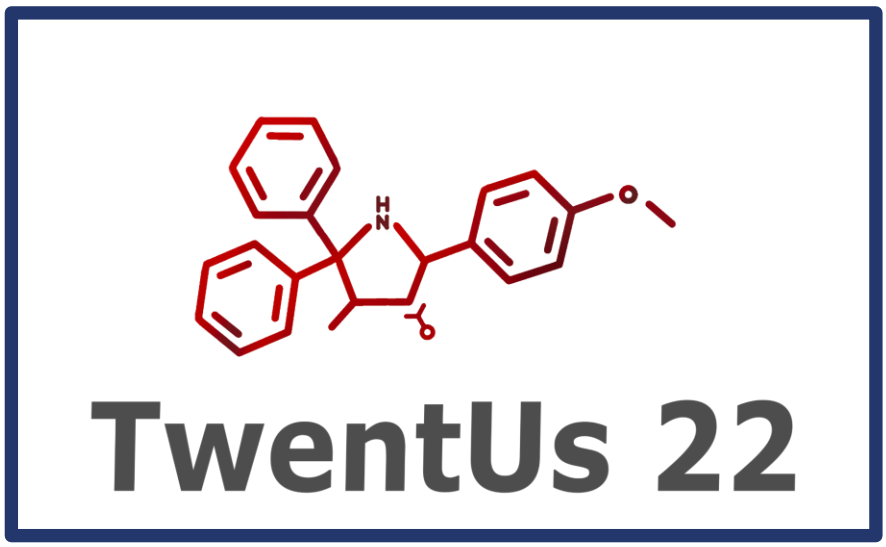
Team Results Document



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SensUs

1. Abstract

TwentUs 22 presents an optical biosensor for interleukin-6 (IL-6) detection via microring resonators. We have envisioned a development plan in collaboration with industry. More precisely, we have adapted an instrument from Delta Diagnostics using Lionix photonic chips functionalized by Xantec with hydrogel coatings. Moreover, we have added an amplification step. Early sepsis diagnose is reliant on the detection of extremely low IL-6. Our experiments targeted the measurement of 100ng/ml of IL-6. This instrument has an automated chip alignment feature and a software application with a graphical user interface (GUI) and data visualization. The specifications have been drawn based on the feedback that we have received from health professionals, caregivers that deal with patients with sepsis diagnose, technicians and a chief technical officer of high-end technology supplier companies. For the clinical translation we have identified nursing homes and general practitioners as market entry. This will allow to early identification of sepsis while offloading hospitals. An important feature is that our sensor can adapted to detect more than one marker, thus with the proper protocol (for example, for IL-8 and MCP-1); and a multiplexing chip, various markers could be detected, diversifying the market while reducing the cost and waste of each experiment.

2. Biosensor system and assay (max. 2 A4)

2.1. Summary of the system

The biosensor presented by the TwentUs'22 team is an optical sensor. Our biosensor makes use of hydrogel-coated chips, with antibodies immobilized onto the hydrogel. The antibodies are able to capture interleukin-6 (IL-6) from the plasma, which allows for the measurement of the concentration of IL-6 by a shift in refraction index. The main sensing devices in our sensor are integrated photonics (*i.e.* optical chips) with micro ring resonators (MRR) with silicon nitride waveguides. MRR chips confine light at the waveguide core with the surrounding (sample-containing) medium[2] —in this case a surface with IL-6 containing blood plasma. At this point of the waveguide, between the circular path of the cavity, an evanescent field extends into the surrounding region such that the *local optical properties* are sensitive to binding interactions at or near the surface (Figure 1a)[2][3]. The most relevant optical properties in this case are resonant wavelength, wavelength traveling in the waveguide, and the refractive index. Micro rings behave similarly to capacitors as they resonate only at a specific wavelength. Thus, once the light of the resonant wavelength is coupled in the waveguide it builds up an intensity while doing multiple loops in the ring and forces binding reactions of the target molecules (Figure 1b)[4]. Therefore, once a target analyte settles near the micro-ring surface, the refractive index, and the resonance properties of the micro rings are altered. The shift in resonance wavelength (*i.e.* frequency) can be determined by monitoring the optical spectrum of the output waveguide.

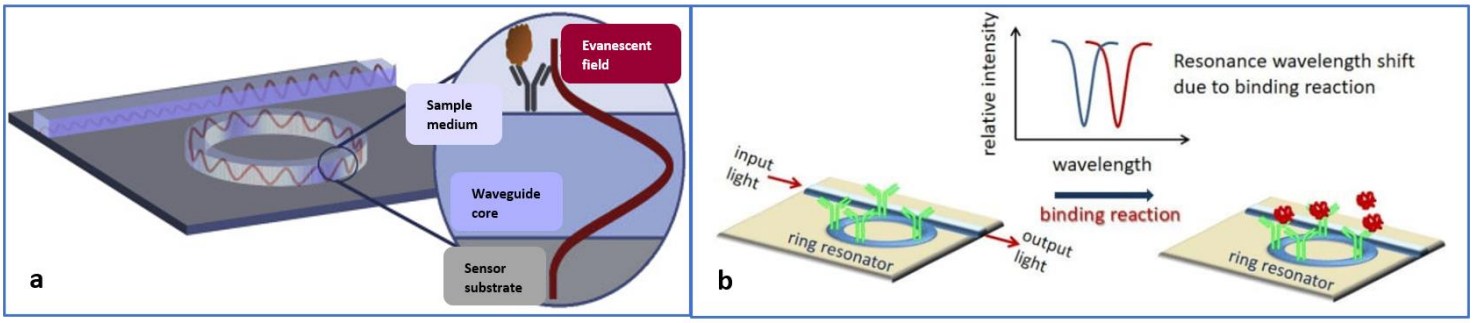

Figure 1. Overview of chemical and biochemical sensing using micro ring resonators **a**. The evanescent wave extending from the wave bus boundary samples the local refractive index[2], **b.** Alteration of the resonating wavelength of the MRR due to the protein binding [4].

2.2. Hydrogel coating and functionalization

The biosensor uses hydrogel coated chips, which are supplied by Xantec. The MRR chips, specifically HC30M, contain a polycarboxylate layer without hydroxyl groups, which is shown in Figure 2A. This prevents the common side effect that the gel interferes with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide (EDC/NHS) esterification by forming crosslinks. The chips are fabricated with layers of Si (i), SiO2 (ii) and Si3N4. These are coated with a layer of 30 nm polycarboxylate (iv).

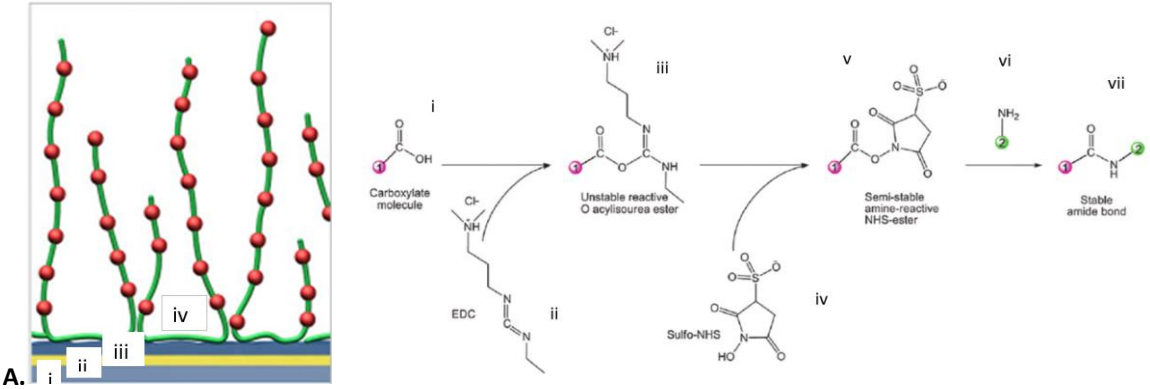

Figure 2(A). The composition of a hydrogel coated chip by Xantec, HC30M, obtained from [5]. **2(B).** Schematic representation of the EDC/NHS activation mechanism, edited from [6]

2.3. How antibodies bind to the hydrogel and chemistry behind

The anti-IL-6 antibodies are not directly bound to the chip. As shown in figure 2B, the polycarboxylate on the hydrogel coated chip (i) interacts with EDC (ii) to form a complex (iii). These make space for (sulfo-)NHS (iv) to replace the EDC that has bound to the hydrogel (iii), by esterification. The formed complex (v) is reactive with amines on antibodies (vi), causing the anti-IL-6 to bind to the chip with an amide bond (vii) and allow for measurement with the chip.

2.4. Amplification step

To perform a more accurate measurement, the acquired signal can be enhanced making use of an amplification step. For amplification, multiple substances can be used, including antibodies, titanium oxide (TiO2) beads and polystyrene beads. The antibodies can bind to the IL-6 that has been bound to the chip. However, this should be more reliable when using compatible antibodies. According to the supplier's specifications, L395 anti-IL-6 is the most compatible secondary antibody for the primary antibody L152 anti-IL-6 [7]. When using nanoparticles (either of the beads), an even higher signal is expected to appear as it increases the refractive index even more. For nanoparticles to bind to IL-6, the nanoparticles need to be coated with the formerly mentioned secondary antibodies.

2.5. Cartridge technology

For the experiments, our team worked with an instrument provided by Delta Diagnostics (see Appendix B) during the development phase. Our setup uses a microfluidic system connected to a software-controlled pressure source (Appendix B-a). The pressure source is connected to the sample tubes (Appendix B-b), so that by regulating the pressure the flow will change accordingly. A valve (Appendix B-c) connects the tube selected via the software to the second valve (Appendix B-d), where the user chooses whether it goes to a waste container (Appendix B-e) or through the chip and then to a different waste container (Appendix B-f).
For the experiment, the chip is placed manually and then aligned automatically by an optical device complemented by special algorithms for distance recognition. The tubes containing samples/solutions are also changed manually. Before the measurement starts the microfluidic system is primed with phosphate-buffered saline (PBS) by connecting the PBS tube to the system, so it goes through the flow cell over the chip and then to the waste bin. The microfluidic system will follow a protocol entered via the user interface, which makes it easily adaptable. This protocol includes a regeneration step between each measurement to reduce waste and lower the time for changing the chip for the next test. After regeneration, the whole system is cleaned with RBS and then MilliQ, to make sure there will be no cross-contamination in the next measurements.

2.6 Reader instrument and user interaction

Commercial prototype and its internal hardware, components and workings are proprietary. Nevertheless, the prototype uses the MRR measuring technique mentioned above that has been envisioned by the team. An implementation of the sensor in the medical field will be based on the core functionalities of this prototype. The sensor consists of 3 parts: a photonic chip with a specific coating for IL-6; a photonic MRR chip to acquire the signal and a fluidics system that controls the flow of solutions, including the blood sample, through the system. The solutions will be provided in packages such that they are easily replaced by the user. The product that will be used in the medical field will need a user interface that determines and communicates the severity of sepsis connected to a fluidics system that works autonomously with the measuring device. Depending on what technology is more convenient for medical workers (microfluidics or regeneration protocol), the software would be integrated together with fast regeneration protocol for the chip and passive microfluidic (like in a COVID test), in case of disposable.

With our approach, we aim to provide a biosensor that can be operated by regular medical personnel. An analyst or chemist would only be required sporadically to perform maintenance on the product. Therefore, we aim to provide a fully closed system that requires minimal user interaction. To use the product, one provides the blood sample to the machine. The preparation of the sample (filtering), running chemicals such as buffer solution and tying the response of the measurement to the severity of Sepsis in the patient will be done autonomously. The tasks of the user are to start certain protocols within the product, such as cleaning the chip at the end of the day and starting a measurement. Apart from the measurement itself, the software will include an administrative system which could potentially be linked to the institution's digital patient system.

3. Technological feasibility

The use of an optical biosensor, functionalized with antibodies on a hydrogel coated chip to detect a biological compound is a proven concept, including IL-6 detection [4]. However, the sensor's potential to detect sepsis is reliant on its detection range, as the concentration of interleukin-6 in a healthy person is extremely low. As shown in Figure 3, the measurement of 100 ng/ml IL-6 has been attempted. In this measurement, prior to the visualized results, EDC/NHS was used to activate the hydrogel coated chip, followed by the addition of L152 antibodies and 1:10 plasma with washing steps in between. Though the wash after the amplification step indicates an increase in signal compared to the wash after IL-6, it is not as high as desired. Therefore, alterations in the bead preparation are necessary. No proof of an increase in signal has been produced, but these alterations should be able to indicate lower concentrations of IL-6. Figure 4 (will be further worked out) visualizes a short-term measurement, in which multiple samples - 1, 10, 100 and 1000 pg - have been measured in rapid succession. The analysis of the results are on-going.

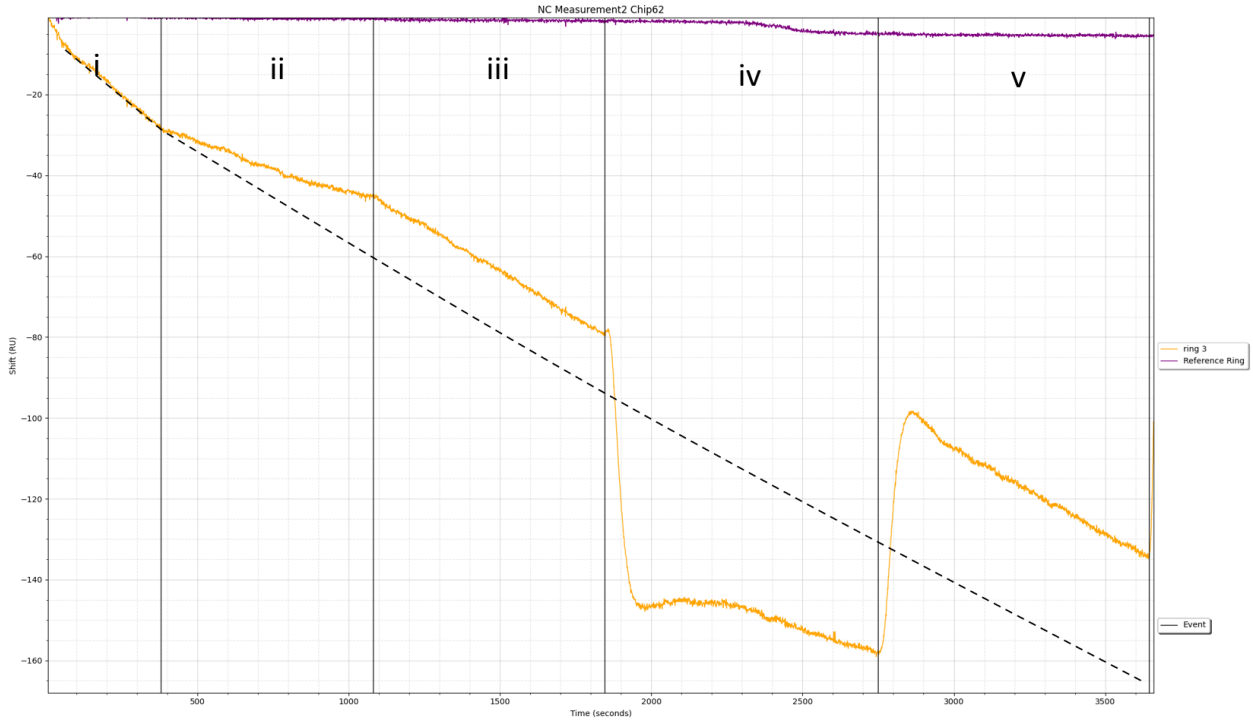

Figure 3. IL-6 measurement acquired from the Delta setup. Results include a baseline with PBS (i), 100 ng/ml IL-6 (ii), a PBS wash (iv) amplification with antibodies (iv) and a PBS wash (v).

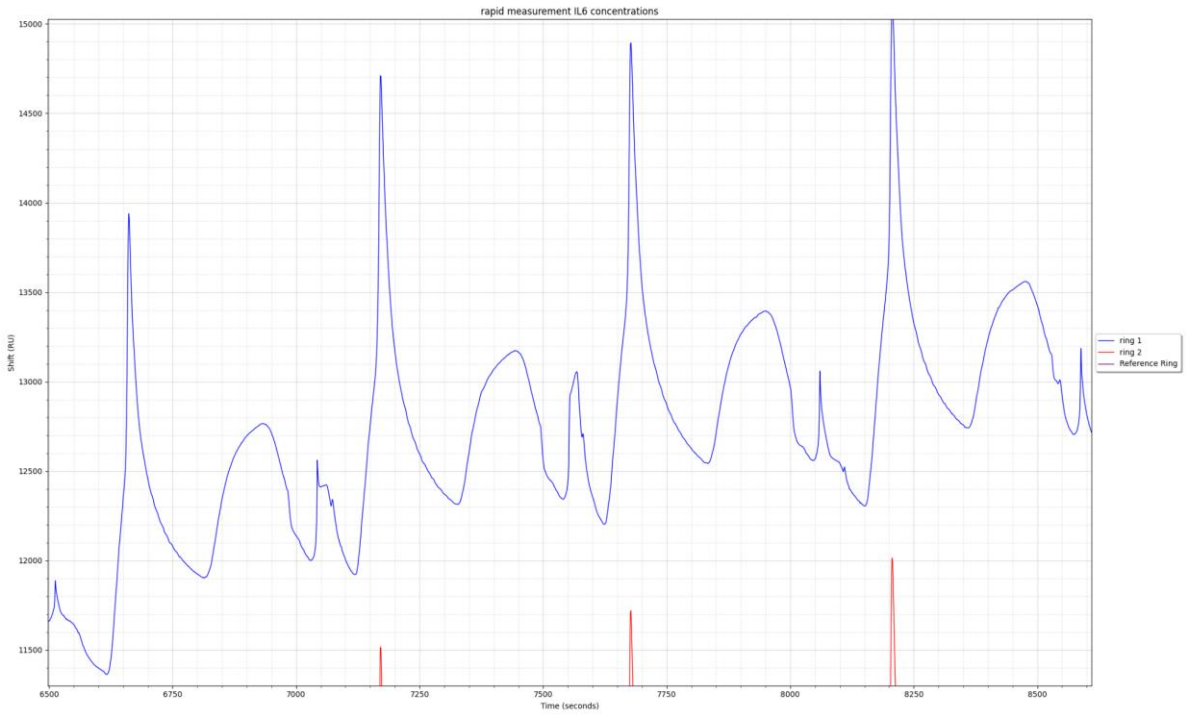

Figure 4. IL-6 measurement acquired from the Delta setup. The results have yet to be analyzed, but for now this is what is presented in this draft.

Considering the local optical properties of the MRR, the resonance condition is established based on a constructive interference condition at the junction between the coupling waveguide and circular path of the cavity [2][3]. This condition is defined by:

$$m\lambda = 2\pi r n_{eff} \qquad (1)$$

where m is a non-zero integer, λ is the wavelength traveling in the waveguide, *r* is the radius of the cavity, and n_{eff} is the effective refractive index. If the radius of the cavity and *m* (determined by setup) are constant, a change in the wavelength is directly and specifically influenced by the refractive index. The refractive index is in turn influenced by the amount of material that sticks to the hydrogel layer of the chip, as well as by any changes in concentration, temperature, and pressure in the bulk. If all other conditions are constant or a reference ring is used, a change in the wavelength can be directly related to the amount of material that is stuck to the sensor ring. As the chip is coated with antibodies against IL-6, the change in wavelength can directly be linked to the amount of IL-6 that is bound by the sensor. Hence, if a set amount of fluid with IL-6 is passed over the sensor, the wavelength shift indicates the concentration of IL-6 in the sample, and as such provides an indication for sepsis.

4. Originality

4.1 Team captains' statement

As a team, we decided to work with optical micro ring resonators (MRR) also using molecular surface functionalization to detect IL-6 in blood. While the instrument we decided to use for the competition is a prototype provided by Delta Diagnostics, our team researched how to adapt the device to the problem, and how to introduce the concept in a medical context. The current measurements are done with one-channel quvet. However, this could potentially be upgraded to a two-channel version for a context outside the SensUs competition, which would allow parallel measurements (IL-6 and negative control on the same chip) and therefore save time.

The flow control system we used was developed at Saxion University of Applied Sciences and tested to our setup. Because this system suited our experiment, no modifications were needed.

For the chips' coating protocol, the antifouling layer was developed by the team members, who consulted our supervisor Nico Overeem to develop it. In addition, a new protocol to detect the IL-6 in the sample was created by the team. This protocol included an innovative regeneration step which would reduce the chip waste and therefore the costs. It will also save time as there would not be a need to realign the chip for each experiment.

Onto the user interface design, the software was created via Adobe XD by our team members. The software was designed as a tablet prototype, and its most outstanding features are that it doesn't allow the user to perform any tasks that could disrupt the measurement unless it's completed or aborted. All the results include a confidence rating, and instead of showing the completed percentage it shows how long each measurement will take. In addition, it is accessible for colorblind people. Also, our team did intensive research of the healthcare market, interviewing healthcare workers, patients and different companies/stakeholders in this sector for feedback collection. It's because of our own research, that we concluded that this kind of biosensor would be more helpful for people at risk for sepsis outside of the hospitals (e.g. at home or nursing homes).

4.2 Supervisory team statement

After completing student recruitment, we provided the team with seed funding support and connected them with several groups at the University of Twente and Saxion University of Applied Science. After exploratory brain storming options, the team decided to work on the solution described above. The plan was guided and seconded with the supervisory team, and the team worked independently for the development.

5. Translation potential

5.1 Business Model Canvas

Our product provides a fast diagnosis of sepsis by blood sample, the focus of our value proposition is early detection. Through our market analysis we have concluded that nursing homes benefit most from our product. Elderly people make up the majority of sepsis cases and nursing homes often lack the expertise/equipment to consistently diagnose their patients with sepsis. Throughout the project we have acquired institutional and commercial contacts, both instrumental to our success. Our sponsor, MESA+, is a nanotechnology institute which has provided us not only with the funding needed for our project, but also the expertise within the institute and their network.

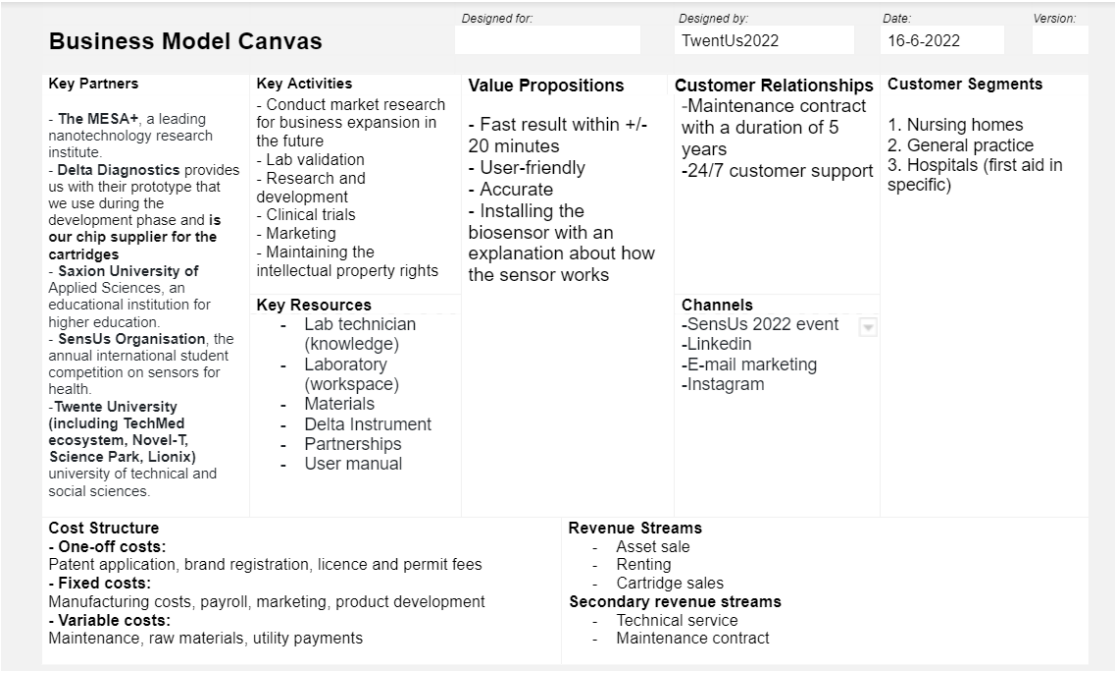

Figure 5. Business Model Canvas

5.2 Stakeholder desirability

In the exploration phase of our market analysis, we have consulted health care professionals that were familiar with sepsis. Microbiologist B.C. van Hees (Appendix D), ICU nurses G.H. Wesselink and S. Boes (Appendix E) and Intensivist B.P.X. Grady (Appendix F), have provided information on the current method of sepsis diagnosis. Using either SIRS or qSOFA, hospital staff can diagnose sepsis through the patient's symptoms. If sepsis is suspected, peripheral blood cultures, sputum cultures and urine cultures are immediately taken. **This is a time-consuming process and fairly expensive because of the many steps and materials required to do so**. **Time is a critical factor with regards to the survival chance of the patient. The results of a blood culture can take up to 48 hours, whilst the patient has an average decrease in survival chance of 7.6% per hour (K. Chun *et al.*)**. To fight the disease, broad spectrum antibiotic treatment is immediately started after the samples are taken. However, a narrow-spectrum antibiotic treatment (significantly more effective) can only be started once the origin of the sepsis is identified (results of blood culture).

We have designed the product to be used by general nursing staff, not just trained analysts. As such, we have put special care in making the product user friendly and intuitive (Appendix J). If the nursing staff has a reason to believe a patient might have sepsis, they can independently take a blood sample from the patient and test it using the product. **Within 20 minutes, the results of the test will be available to the nursing staff and further actions can be taken.** With an early detection (asymptomatic), the patient is not that sick as of yet and treatment will be more successful. Since the nursing staff is testing asymptomatically, they will be testing a significant amount of healthy people. Therefore, we have chosen a reusable cartridge design. Using and regenerating the same measuring chip for multiple tests lowers the cost per test for the customer. Furthermore, the chip is split up into four channels allowing for a higher capacity (4 tests being done simultaneously), as well as getting even more mileage out of a single chip. The low cost per test and high capacity will allow the customer to test freely across their patients.

5.3 Business feasibility

The resources, skills and expertise required for development, fabrication and scale-up of the biosensor include laboratories, Delta instrument, raw materials, chemical knowledge, capital resources for assembly of the biosensor and lab validation.

We are mainly sponsored by MESA+, institute/University of Twente, who supports the team by funding and giving lab access, providing important contacts and giving R&D support. One of the partners of TwentUs'22 is Delta Diagnostics, they are a company that is developing a similar product. They have provided us with their research prototype for us to use in the competition. In addition, they have answered all our questions on the technical details and application of such a device. Through our collaboration we have explored the potential of MRR technique in the context of sepsis. Delta diagnostics has supplied us with the chips needed for measurements through their partners. These chips are made by Lionix and coated by Xantec. Our plan is to realize the product with the support and expertise of our partners, to apply the collective knowledge to the context of sepsis diagnosis.

In the first stage of our product, we will market the product as an early detection system for sepsis to nursing homes. Through social media, email marketing and events, we will gradually expand and increase our network by reaching out to key opinion leaders. The instrument will be a substantial initial cost for our customers, as such we will provide payment plans and options for renting the device. The possibility of marketing the product as a shared instrument between nursing homes in the same region is to be explored as well. This would spread the risk of investment across multiple customers and help spread our brand across multiple customers at once.

The revenue stream of the product consists of the instrument itself, providing the measurement chips, providing the chemicals to the customers and maintenance. The measurement chips are functionalized specifically for the product and they need to go through a strict quality control as they have to be calibrated for use in the factory. The chemicals for the machine come in pre-packaged kits which are easy to apply to the machine in order to refill its fluids. Maintenance to the product, in case of an error, will be provided by company technicians. Having multiple streams of revenue provides more stability to the company as the product still generates income after it is sold.

In the second stage of the product, after having acquired funding from the success of the first stage, the product will not only detect sepsis itself, it will detect the cause of the sepsis. The biggest advantage of using micro ring resonators is its generality. With a different functionalization of the chip, a different chemical can be tested for. Therefore, with limited alterations to the first product, this product could test for a panel of chemicals that indicate the origin of the sepsis. This product would be marketed for hospitals, as these would benefit greatly from reducing the time to diagnose the origin of sepsis to only 20-30 minutes (opposed to a potential 48 hours). We have conducted research with medical staff that indicates that such a product would revolutionize the care for sepsis and that such a product would be in high demand.

5.4 Market description

We quantify the market by how many people are affected by Sepsis. The number of people that have faced sepsis worldwide in 2017 is 48.9 million people with around 11 million casualties. These 11 million casualties contribute to 19.7% of total deaths worldwide [9]. In Europe, the death toll is estimated to be 680,000 per year, but because of the lack of accurate records and reporting, this is certainly an underestimation. In these cases, older people are the most affected population group[10]. According to ZorgkaartNederland, there are currently 2356 nursing homes and care homes in the Netherlands, which currently account for the care of 115.000 people [9]. Germany currently has a number of 15.000 nursing homes [11].

Currently sepsis is diagnosed as follows: When a patient arrives at the first aid with the suspicion of sepsis, during the first hour the patient will be analyzed on the sepsis criteria and the following 30-60 minutes for retrieving the result of blood test and a special score. This is done based on the qSOFA score (Quick Sequential Organ Failure Assessment), which uses three criteria(see Appendix 3)[12]. Accordingly, when a patient meets two criteria or more, the patient has a septic profile.

Our group will focus on preventing hospitalizations of people suffering from sepsis by developing a biosensor that detects sepsis earlier in people than the current method of diagnosis described in the paragraph above. During an interview with B. van Hees (see Appendix 4), we concluded that such a biosensor would be of most

value in nursing homes. This is because when people with a septic profile arrive at the first aid at the hospital it is not difficult to diagnose sepsis. Most of the time the patients meet two or more criteria from the qSOFA and therefore antibiotics will be given immediately whether there will be a biosensor or not. However, it will always be of added value to detect sepsis in an early stage before the patient becomes really sick and arrives at the hospital (see Appendix 4). The biosensor could be of added value because it can be difficult for them to decide whether they send a patient to the hospital or not. A physician can analyze a patient on clinical presentation but has much fewer tools than a hospital. Therefore, a biosensor that can detect sepsis within a small period of time would be of added value (see Appendix 4).

Most sepsis cases start occurring in people older than 40 years. However, death caused by sepsis also occurs in newborn babies (<1year), most often due to a bacterial infection, with a strong increase after the age of 60 (Appendix 7)[13] However, this information should not exclude the urgency that there is also a need for a rapid diagnosis of sepsis in people in other age groups, even if this is less common.

After researching some figures known about nursing homes in the Netherlands and Germany, which are described in the first paragraph, we came to the conclusion that there is a large market here. Our target market entry will therefore be the Netherlands and Germany. Nursing homes are only expected to increase due to heavy aging, which will make the market even bigger. For an overview of the forecast of aging in the Netherlands (see Appendix 8)[14]. Also, the demand for our biosensor is more important in a nursing home than in the other markets (hospital and general practice) to detect early detection in order to start treatment earlier and increase the chance of survival for the person (see Appendix 4).

5.5 Revenue prediction

We have 2 revenue streams, one for the instrument and another one for the chips. The market for the Netherlands consists of 2365 care homes and the market for Germany consists of 15.000 care homes. As follows we details our revenue prediction. We estimate the cost per biosensing setup, and a market penetration for the Netherlands and Germany for 5% to 10% for the first year after launch (1000 and 2000 homes respectively). All calculations exclude VAT.

Part cost	**Instrument**
Fluidics	**1200€**
Laser	**750€**
Alignment	**400€**
Optical sensor	**600€**
Case, screen etc.	**1000€**
Total cost instrument	**3950€**

	Costs	**Sell price**	**Markup**	**Frequency**
Instrument	**3950€**	**7000€**	**77%**	**1***
Chip (+functionalization)	**40€**	**100€**	**150%**	**As needed by demand****
Chemicals	**50€**	**100€**	**50%**	**1 per quartile**

*We will consider other revenue models such as monthly subscriptions including technical maintenance or leasing options, to expand the profit and positive cash flow over time.

**Estimated 5 tests/month for each elderly home.

Gross profit prediction (one off sale for the instrument, yearly forecast for the chips). Profits on chemicals are excluded for clarity (might be included in the price per chip later). With 5 test a month at 60€ of profit per chip, each elderly home provides 3600€ gross profit:

Profit prediction	**Per customer**	**5% of market NL+DE, 1000 homes (rounded)**	**10% of market NL+DE, 2000 homes**	
Profit instrument	**3950€**	**3950000€**	**7900000€**	**Profit instrument**
Profit per chip	**60€**	**3600000€**	**7200000€**	**Profit yearly**

6. Team and support

6.1 The TwentUs'22 Team

Anton Zuboskyi	One of the team leaders. Main role - team organization, weekly agendas, communication with stakeholders and involved parties. Participated in lab work: measurements + sample preparation.
Bas Bernasco	Mainly engaged in the business part and thus responsible for the translation potential part.
Marell Slag	Mostly involved in the biomedical particle, literature research on the sensor/ sepsis/ IL-6 and the social media part
Michelle Tijdink	Mostly involved in the biomedical particle, conducting interviews and literature research on the sensor/ sepsis/ IL-6
Niek Schepers	Mainly responsible for all laboratorial processes, including making the protocols, preparing samples, handling the equipment to acquire data, distribution and interpretation of results. Supervision of the rest of the team in the lab.
Olga González Gimeno	Engaged in the literature research on the sensor/ sepsis/ IL-6. Participated in lab work: measurements + sample preparation. Social media responsible.
Robin Broekman	One of the team leaders. Main role - team organization, weekly agendas, communication with stakeholders and involved parties. Participated in lab work: measurements + sample preparation. Made the end-user interface and collaborated on a hypothetical product concept.
Ruth Wesselink	Most involved in the biomedical particle, conducting interviews, literature research on the sensor/ sepsis/ IL-6 and the social media part
Wessel Meeringa	Most involved in the biomedical particle, conducting interviews and literature research on the sensor/ sepsis/ IL-6

6.2 People who have given support

Dr. ir. Pep Canyelles Pericàs	Team supervisor, leading role in recruitment, helped with organizational staff, mentored the team, assisted to get funding from partners and forging parnerships; and oversaw the team progression.
Dr. ir. Nico J. Overeem	Team supervisor, helped with surface chemistry processes, amplification step, main protocol development and lab practicals.
A. Ymeti	Project leader, researcher Expertise: Physics, nanosensors, point-of-care devices, business
M.L. Bennink	Professor (lector), lecturer Expertise: Applied physics, biophysics, nanotechnology, project management
M.J. van Rossum	Project leader, researcher, lab manager Expertise: Biology, Life Sciences
Marko Blom	Chief Technology Officer at Micronit Microtechnologies. Expertise: Microfluidics, Integration of sensors, Medical Devices.
Ziekenhuisgroep Twente (ZGT)/ Deventer Ziekenhuis (DZ)	Hospitals. We were given the opportunity to conduct interviews with healthcare professionals.
Tessa Bouwmeester	Lab researcher, helped with protocol for surface coating and lab experiments.
Ruben van Harmelen	Product application specialist at Delta Diagnostics. Helped with the technical details of the prototype that is used, as well as giving consultancy on the potential of the measuring method and providing results from their own testing with the prototype.

6.3 Sponsors

Mesa+ Institute / University of Twente	The MESA+ Institute is a leading nanotechnology research institute. They focus on societal challenges in four application areas: Health, AgriFood & Water, Security, and Energy & Sustainability. With their research, they contribute to a fair, sustainable and digital society.
Delta Diagnostics	Delta Diagnostics envisions a world in which new multiplexed and sensitive assays can be developed rapidly and at low cost. They are providing their prototype to the team.

7. Final Remarks

Also we would like to thank Mesa+ of the University of Twente for funding our project & Delta Diagnostics for providing us usage of their prototype of the biosensor we are using at the competition in Eindhoven.

8. References

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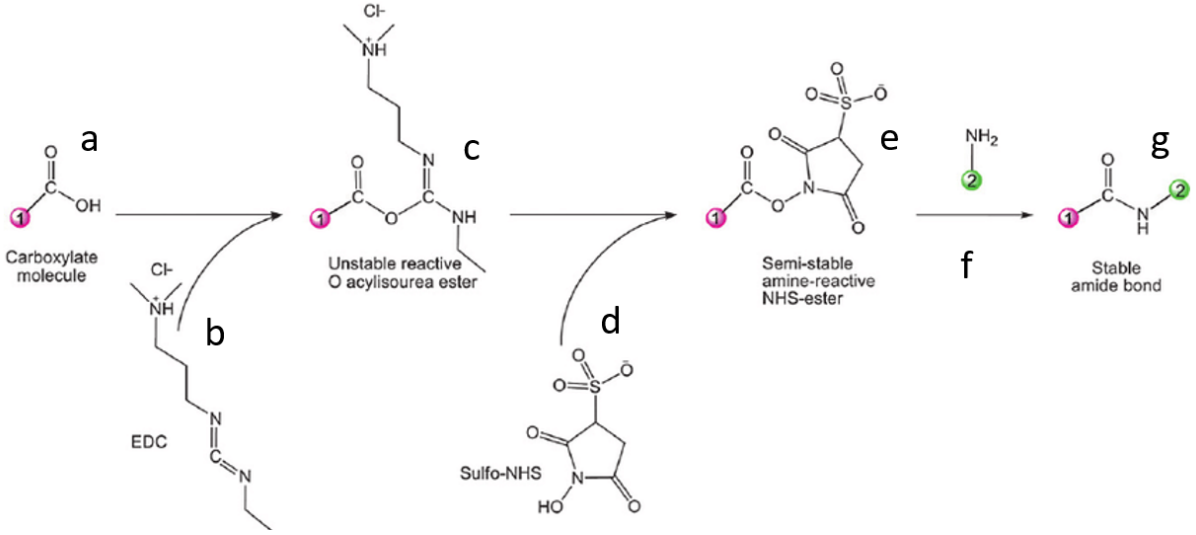
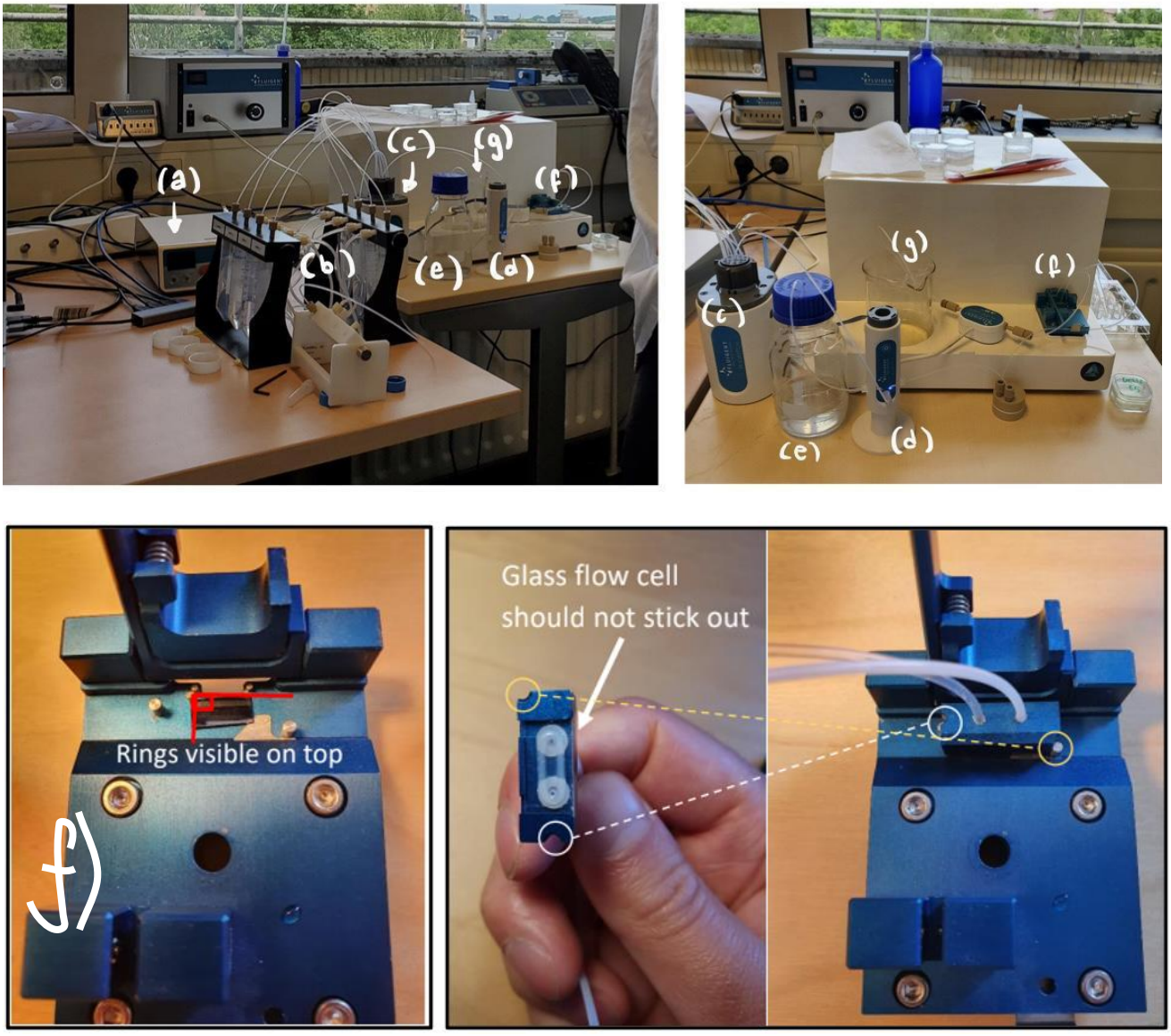
9. Appendices

Appendix A: EDC/NHS conjugation



Appendix B: Cartridge technology - experimental setup



Experimental setup with Delta MK4 Analyzer. ***a****. Pressure source* ***b****. Samples' tubes* ***c****. M-switch* ***d****. 2-Switch* ***e****.Waste bin* ***f****.Chip holder with cuvette from microfluidics system* ***g****. Waste "bin"*

Appendix C - "*SIRS criteria and qSOFA score*"

Table 1. *SIRS criteria and qSOFA score*

SIRS criteria (≥ 2)	Body temperature > 38.0 °C or < 36.0 °C
	Heart rate of > 90/min
	Respiratory rate of > 20 breaths/min or $PaCO_2$ of < 4.3 kPa
	White blood cell count of < 4000 cells/mm³ or > 12,000 cells/mm³ or > 10% immature bands
qSOFA score (≥ 2)	Respiratory rate ≥ 22 breaths/min
	Systolic blood pressure ≤ 100 mmHg
	Altered mental state

SIRS = systemic inflammatory response syndrome; qSOFA = quick sequential organ failure assessment.

Note. "Reprinted from "Classifying sepsis patients in the emergency department using SIRS, qSOFA or MEWS" by S.W. van der Woude; F.F. Doormaal; B.A. Hutten; F.J. Nellen; F. Holleman, 2018 (https://njmonline.nl/article_ft.php?a=1977&d=1301&i=215).

Appendix D - "Interview with microbiologist and medical director"

Interview B.C. van Hees

MD, Clinical Microbiologist and medical leader at Deventer Ziekenhuis
This interview with B.C. van Hees was held on the 25th of April 2022 via Teams.

How do you as a professional deal with sepsis?

1. Prevention
   - Enforcing measures that can be taken to prevent sepsis
   - Hygienics
2. Diagnostics
   - When a patient is suspected of having sepsis, blood cultures will be taken. Besides general blood cultures, local blood cultures will also be taken from places where the infection is thought to be.
3. Treatment
   - When the patient has a septic profile and all the blood cultures have been taken, a treatment policy will be carried out. Thereafter, a set of antibiotics will be chosen and given to the patient.

When is a patient diagnosed with sepsis?

1. Clinical presentation of a patient (from the outside)
   - When a patient arrives at the first aid with the suspicion of sepsis, during the first hour the patient will be analyzed on the sepsis-criteria;
   - This is done via the qSOFA score, (also known as quick SOFA) a bedside prompt that may identify patients with suspected infection who are at greater risk for a poor outcome outside the intensive care unit. It uses three criteria. When a patient meets two criteria or more, the patient has a septic profile (CRISMA CENTER, CRITICAL CARE MEDICINE, UNIVERSITY OF PITTSBURGH, & UPMC, sd);
   - assigning one point for low blood pressure (SBP≤100 mmHg)
   - high respiratory rate (≥22 breaths per min)
   - altered mentation (Glasgow coma scale<15)
2. Also during the first hour, blood cultures will be taken from the patient and will be analyzed in the lab. The results of the blood cultures are currently available within 4 to 48 hours depending on the amount of the present bacteria. However, when the patient meets two or more criteria from the qSOFA, the patient is already seen as septic and antibiotics will be given.

Constraints in analyzing septic patients

1. Younger people who do not look sick from the outside but are septic.
2. A patient with a septic profile but negative blood cultures.
3. A lack of information about what specific bacteria the patient deals with.
4. Not exactly knowing what specific antibiotics the patient needs.
5. Difficult to find out where the infection exactly is in the patients body.

Is there a specific group of people you see coming in more often with sepsis?

1. People with comorbidities
2. People with diabetes or people under treatment for cancer
3. People with a reduced immune system
4. Elderly people (60+)

However, there is no specific group to designate. People from all ages can develop sepsis. For example, baby's, pregnant woman's, but also young healthy people who develop sepsis from an abscess.

When we as a group want to detect sepsis with our biosensor, what should we look for?

- **Leukocytes** (white blood cells). An increased amount of lymphocytes is usually the result of an infection with a virus and sometimes a bacteria.
- **Thrombocytes** (blood platelets) dangerous when there are too few
- **Creatinine** urine test measures the amount of creatinine in urine. This test is done to see how well your kidneys are working. Creatinine can also be measured by a blood test.
- **Bilirubin -** an elevated bilirubin level in the blood means that the liver is not functioning optimally.
- **Lactate –** increased when a patient has sepsis

Would a biosensor be of added value in the hospital?

It would definitely be of added value to have a biosensor that can detect sepsis in a short period of time. However, such a biosensor would be more valuable in nursing homes or general practice than in a hospital. This is because when people with a septic profile arrive at the first aid at the hospital it is not difficult to diagnose sepsis. Most of the time the patients meet two or more criteria from the qSOFA and therefore antibiotics will be given immediately whether there will be a biosensor or not. However, it will always be of added value to detect sepsis in an early stage before the patient becomes really sick and arrives at the hospital.
For a general practice for example, this biosensor could be of added value because it can be difficult for them to decide whether they send a patient to the hospital or not. A doctor in a general practice can analyze a patient on clinical presentation but has much fewer tools than a hospital. Therefore, a biosensor that can detect sepsis within a small period of time would be of added value.

When a hospital will use our biosensor, what criteria does it have to meet?

1. Easy to use
2. It must be able to be linked to the EPD (Electronic Patient File/Hospital Information System)
3. The result should be clear: what action is needed by what result
4. Dummy-proof
5. Fast

Appendix E - "Interview with ICU nurses at hospital"

Interview G.H. ten Klooster and S. Boes

Intensive Care Unit nurses at Ziekenhuisgroep Twente (ZGT)

All information in this document is conducted from the interview held with G.H. Ten Klooster and S. Boes on the 26th of April 2022 at ZGT Almelo (Boes and Klooster, 2022). Both gave verbal permission to process and share the data obtained by them with third parties.

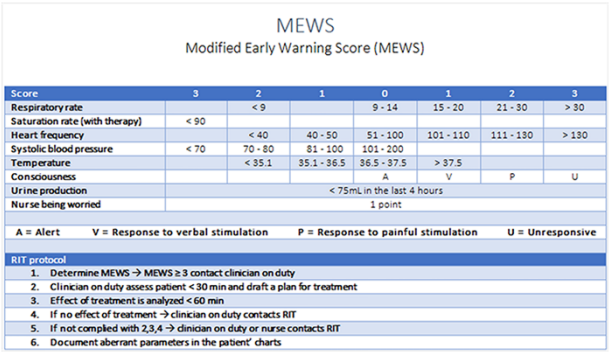
How do you as a professional deal with sepsis?

In an intensive care unit, patients with sepsis or septic shock are treated almost every day. When there is a suspicion of sepsis or a diagnosis, the nurses are responsible for monitoring and treating the patient. The ICU nurses do this by administering prescribed medication and taking lab tests. In addition, they monitor the patient's vital signs very accurately using a monitor.
In addition, they are also responsible for hygienic work. By working hygienically, a sepsis infection can be prevented. Examples include: Sterile insertion of a catheter, prevention of line sepsis, etc.
Boes indicates that sepsis used to be much more common back in the days. The MEWS score is now mandatory at an ER and nursing wards, so a patient with possible septic symptoms is identified much earlier. As a result, a patient can be treated earlier (and faster) and a deep septic shock is becoming less common.

How do you diagnose sepsis?

Within the ZGT, protocols are used. A protocol has also been drawn up for suspected sepsis. The ICU uses the protocol: 'sepsis in the intensive care unit, diagnosis and treatment of'. The aim of this protocol is to establish and treat sepsis in an unambiguous manner.

1. Two scoring systems (MEWS and qSOFA) are used. This allows the clinical symptoms to be scored.



MEWS
Modified Early Warning Score (MEWS)

Score	3	2	1	0	1	2	3
Respiratory rate		< 9		9 - 14	15 - 20	21 - 30	> 30
Saturation rate (with therapy)	< 90						
Heart frequency		< 40	40 - 50	51 - 100	101 - 110	111 - 130	> 130
Systolic blood pressure	< 70	70 - 80	81 - 100	101 - 200			
Temperature		< 35.1	35.1 - 36.5	36.5 - 37.5	> 37.5		
Consciousness				A	V	P	U
Urine production	< 75mL in the last 4 hours						
Nurse being worried	1 point						

A = Alert V = Response to verbal stimulation P = Response to painful stimulation U = Unresponsive

RIT protocol
1. Determine MEWS → MEWS ≥ 3 contact clinician on duty
2. Clinician on duty assess patient < 30 min and draft a plan for treatment
3. Effect of treatment is analyzed < 60 min
4. If no effect of treatment → clinician on duty contacts RIT
5. If not complied with 2,3,4 → clinician on duty or nurse contacts RIT
6. Document aberrant parameters in the patient' charts

MEWS: Modified Early Warning Score. Determines the degree of illness of a patient using 4 physiological findings and one observation.
qSOFA: a score that Identifies high-risk patients for in-hospital mortality with suspected infection. The score ranges from 0 to 3 points. The presence of 2 or more qSOFA points near the onset of infection was associated with greater risk of death or prolonged intensive care unit stay. the score is based on the following three aspects:
   - Low blood pressure (SBP<100 mmHg)
   - High respiratory rate (>22 breath per min)
   - Altered mentation (Glasgow coma scale <15)
2. If sepsis is suspected, peripheral blood cultures, sputum culture and a urine culture are immediately taken afterwards. In addition, low-threshold diagnostics such as punctures and drainage are also performed, in order to find the source of the infection.
3. As soon as the cultures have been taken, antibiotics are immediately started. Most of the time with broad-spectrum antibiotics or antibiotics guided by previous cultures.

Constraints in analyzing septic patients

1. Younger people who do not look sick from the outside can be septic.
2. the matter of time. You want to treat patients as quickly as possible with the right antibiotics. The longer a sepsis infection exists, the sicker a patient becomes and the more organ function has to be taken over by machines, etc.

3. Difficult to find out what the source of infection exactly is in the patient's body.
4. A broad spectrum antibiotic is first started, only after the results are known, a specific antibiotic can be started.

Is there a specific group of people you see coming in more often with sepsis?

1. patients with a compromised immune system
2. patients on immune-suppressing drugs, such as corticosteroids
3. elderly people
4. patients where vital functions are taken over by machines, such as ventilated patients
5. patient patients on an IV (line sepsis)

Would a biosensor be of added value in the hospital?

Within ZGT, no biosensor is used for the detection of sepsis at the moment.
a biosensor as should be designed for the SensUS 2022 competition:

1. No added value on an ICU.
2. Value in other nursing departments/ER.
    a. If it can prevent or reduce treatment, it's a good added value
    b. If the nurses don't feel right about the situation of a patiënt - easy and quick test to diagnose or rule out sepsis.

If the biosensor can also indicate which bacterium causes the sepsis, this is of added value on an ICU.

Geerrinda also indicates that a biosensor could possibly be a solution for home care or a nursing home. In this way, hospitalization can possibly be prevented by proper treatment at home. Or, for example, people who no longer want to go to hospital. With the help of a biosensor it is possible to look for an appropriate treatment at home.

In addition to determining values from the blood, the nurses indicate that the human factor is also very important. By this she means that the image a nurse gets of a patient also plays a role. For example, it may be that the values in the blood are good, but the patient looks very sick. Then it must be treated properly.

When a hospital will use our biosensor, what criteria does it have to meet?

1. Easy to deploy/ easy to use
2. Fast result
3. Price friendly - not too expensive
4. Specific measurements for specific bacteria so that treatment can be started even faster and the right therapy can be used.

Appendix F - “Interview with intensivist”

Interview Dhr. Dr. B.P.X. Grady

Mr Grady is an intensivist at the ZGT of Almelo.
The interview took place on 26-4-2022

How do you as a professional deal with sepsis?

Daily - many forms of sepsis. Depending on where the bacteria is located (eg urosepsis, lung sepsis, etc.)
Most common form of sepsis in IC ZGT - abdominal or pulmonary
Sepsis is treated by means of antibiotics and a good filling. Sepsis is a guideline, also known as the golden hour, in which diagnosis plays an important role. Sometimes it is important not to act too quickly. Sometimes it seems that a patient has sepsis, but this can also be another condition. However, this is very difficult to distinguish, so that sometimes treatment is started too quickly.

When is a patient diagnosed with sepsis?

Blood cultures in combination with patterns, so you can see that someone has a distributive shock (abnormalities in the lung photo, abdominal pain, low blood pressure, fast heart rate, acidosis, etc.). Early detection is also done by means of the qSofa or early warning score.

Constraints in analyzing septic patients

The most difficult in sepsis is a SIRS reaction → so for example it is an inflammation or infection. It is therefore possible that a patient is given antibiotics without it being necessary. Often antibiotics are also administered with a SIRS. The most important thing is to “fill” the patient and ensure that the tissues are supplied with sufficient oxygen.
The aim is to act as quickly as possible - time can be a disadvantage.

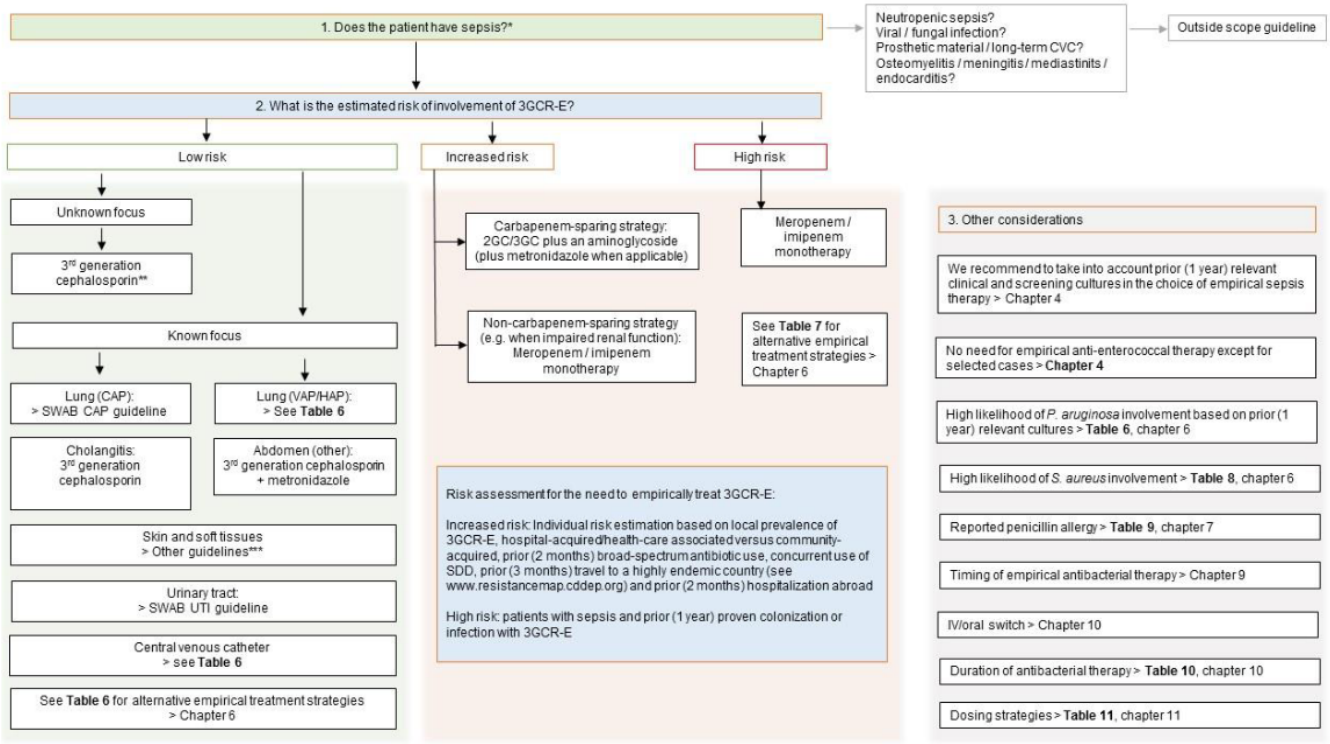


Figure 1. Flow chart of guideline recommendations on empirical antibiotic treatment of sepsis. Retrieved from https://swab.nl/exec/file/download/144

Is there a specific group of people you see coming in more often with sepsis?

Sepsis is contracted daily. More common in older people with fragile health. Or, for example, through the use of medication.
Patients with sepsis from outside the hospital are more common. Within the hospital, it often has to do with abdominal surgery, which sometimes causes abdominal sepsis.

Would a biosensor be of added value in the hospital?

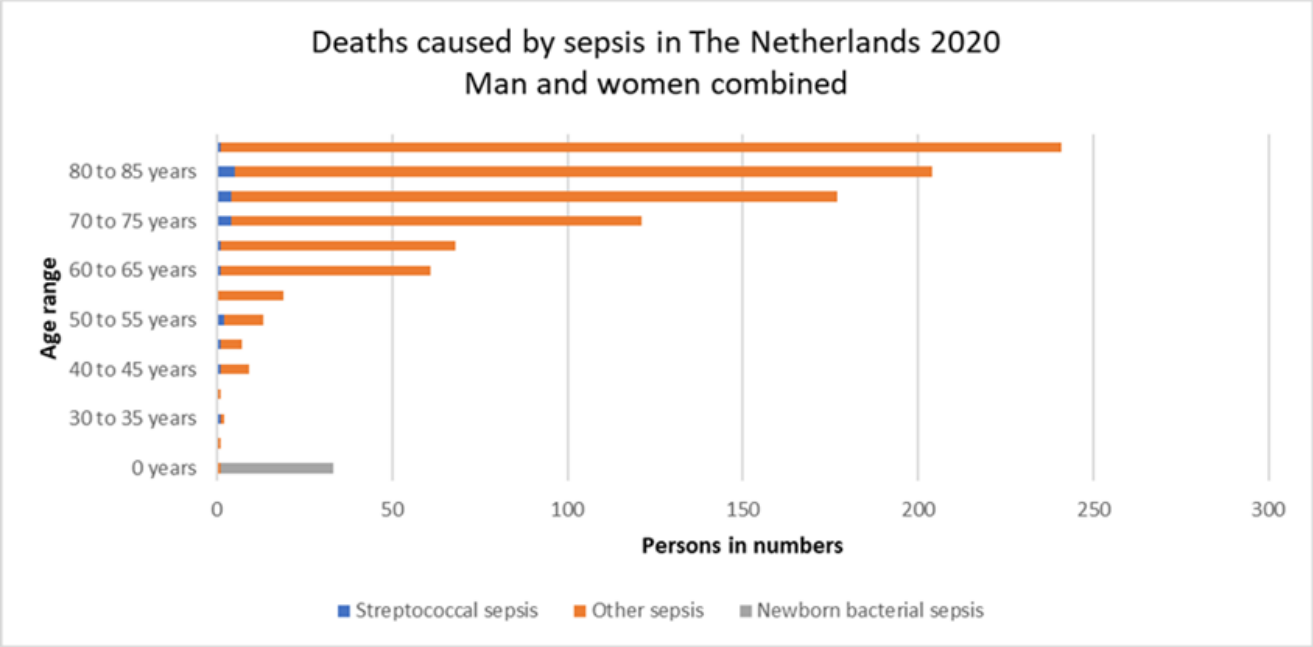
Yes, it has added value. For example, it makes a difference in the unnecessary use of antibiotics. In addition, it also makes a difference in possible resistance that can occur in the patient. A biosensor would offer added value for the early detection of sepsis and the earlier initiation of treatment.
Patient delay (patient arrives late), treatment delay (doctor arrives late), antibiotic engorgement chip (prescribe antibiotics as specifically as possible, to prevent resistance) can be prevented.

When a hospital will use our biosensor, what criteria does it have to meet?

It is important that the biosensor is “dummy proof”. This means that the sensor should be usable by all ages/levels of caregivers. For example, remember that it is shown step by step how the biosensor works.
It must also meet the following requirements:

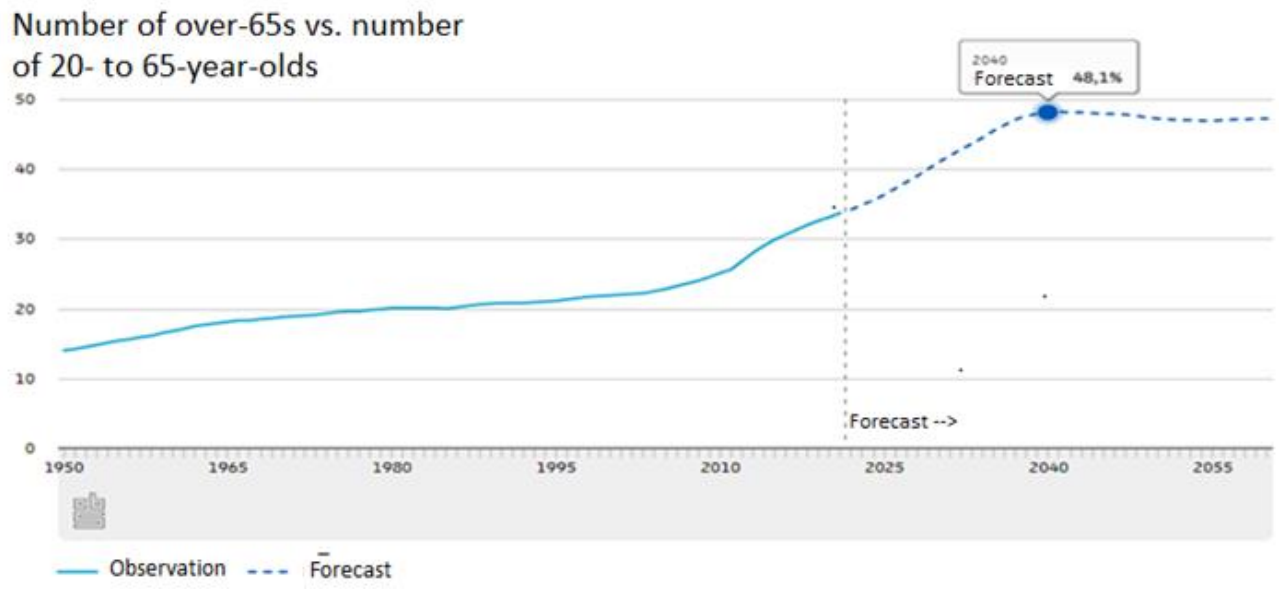
- In addition, the device must not cost too much.
- Sufficient research must be done.
- The device must be validated (which is standard
- Must be demonstrated and independent.

Appendix G - “Death rate from sepsis”



Note. Reprinted from "Deceased; cause of death (expanded list), age, sex," by CBS, 2022 (https://www.cbs.nl/nl-nl/cijfers/detail/7233?q=sepsis).

Appendix H - ”How aged is population in Netherlands”



Note. Reprinted from "How Aged is the Netherlands?" by CBS, 2021 (https://www.cbs.nl/nl-nl/visualisaties/dashboard-bevolking/leeftijd/ouderen).

Appendix I - “Interview with Micronit”

Interview Marko Blom

Marko Blom is the CTO of Micronit. The interview took place on 29-06-2022

If we focus on regeneration and multi-channel MRR chips, what aspects of the cartridge technology should we pay attention to?

The cost per test is a good measurement to pay attention to. Also, you would have to make sure the cartridge is robust enough to be used multiple times. Especially the interfacing between the cartridge and the instrument must be robust. If there is any leakage, you would have a problem.

What would be a good way of finding a suitable price range for the device?

Again, the cost per test is an important metric. However, take a general practitioner's office for example, if the price per test is only 1ct but the initial investment is too high, they will not use it. To give an estimation, 5000-1000 EUR would be doable if the cost per test is low.

What currently developing biosensors would you call promising?

Microtechnology is a good fit for point of care products due to its small size. For example, it is possible to include a laser and detector on the chip, where the fluidics take care of the metering. Additionally, disposable cartridges could use a passive flow, removing the need for a fluidics system entirely. At this point you would hardly need an instrument anymore. With this micro scale you could miniaturize the whole system.

Comments on our use case

In the use case we use a filtration system to filter the bigger components of the blood.
You would need to clean the device/tray quite thoroughly. To bring this solution to the market you would have to prove there is no cross-contamination between the first and second measurement in the same channel. A more common approach is to use disposable filters.

In the use case we use four separate channels which can be used in parallel
It would be quite difficult to align all these channels simultaneously, though it is not impossible.

Appendix J - “Graphical User Interface concept for product”