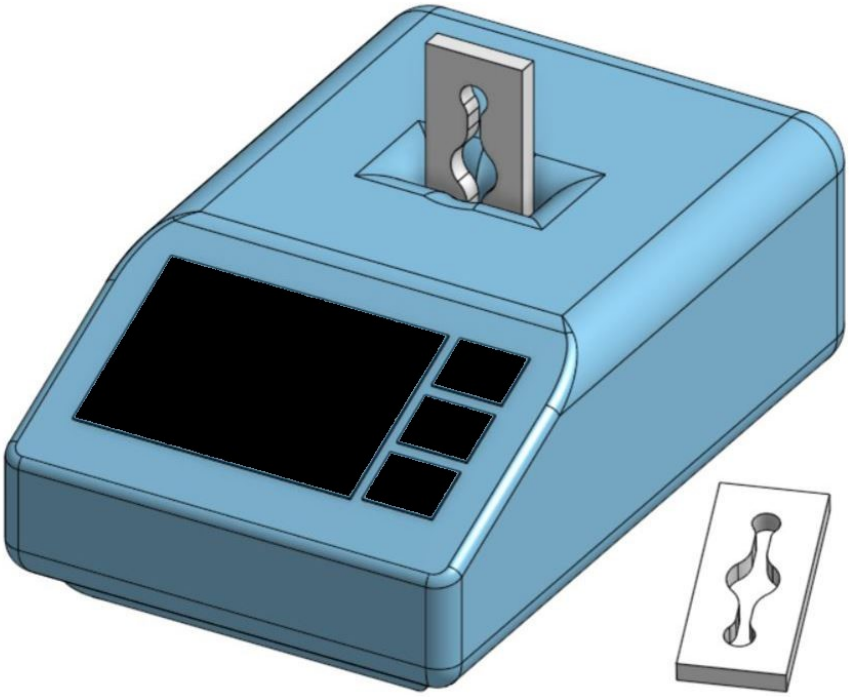
YOUR LABORATORY AT HOME



T.E.S.T. 2017

Team Results Document

Team members: Joost Bergen, Max Bergkamp, Tim Donders, Jamy van Geemen, Lotte Hagedoorn, Gaby van Iersel, Maaike Kraamer, Amy Lucassen, Maartje Pontier, Sophie Roos, Imke van der Schoor and Paul Vernooij

Support: Dr. L. J. van IJzendoorn and M.R.W. Scheepers, MSc

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SUMMARY FOR THE SENSUS WEBSITE

Driven to improve healthcare with innovative technologies, T.E.S.T. has developed a small and easy-to-use biosensor for the detection and monitoring of heart failure. Heart failure is an increasingly common disease in the Netherlands. With around 227.000 patients and 40.000 new diagnoses every year, our biosensor would help out patients in many ways. With an original system and a creative design, our biosensor could deliver fast, reliable and accurate results.

Our sensor makes use of a cartridges in which the blood plasma sample is inserted. The cartridge contains magnetic particles coated with antibodies, which are mixed with the sample. The antibodies bind to the protein NT-proBNP in the sample. With a magnetic field, the motion of the magnetic particles is manipulated and clusters are formed: two particles are bonded by an NT-proBNP molecule. A rotating magnetic field is applied to measure the time-dependent cross-section of the clusters. The strength of the signal relates to the concentration of NT-proBNP.

Our sensor is designed for home monitoring, which is a unique concept on the Dutch NT-proBNP sensor marker. Close monitoring of severe patients will improve the changes of early detection of deterioration and prevention of hospitalization. Our biosensor is your laboratory at home!

1. BIOSYSTEM AND ASSAY

The main goal of the second edition of the SensUs competition is to detect NT-proBNP, a key biomarker for heart failure, as fast and as accurate as possible. In this section, the proposed detection principle is explained and our biosensor system is shown.

1.1 Detection principle

In our system, we will use an opto-magnetic cluster assay to detect NT-proBNP. In this type of immunoassay, the analyte is sandwiched between two different antibodies. The antibodies are attached to superparamagnetic beads. If the NT-proBNP is caught, a cluster will be formed consisting of two beads, with the NT-proBNP in between (see figure 1). These clusters will play a key role in the detection of NT-proBNP.

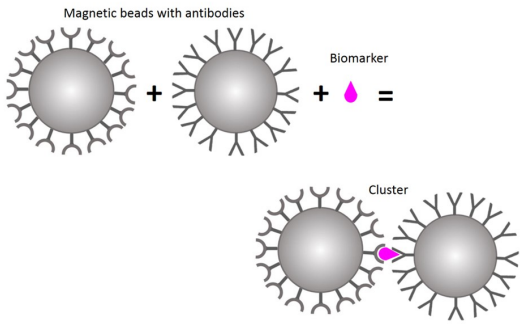

Figure 1: Cluster formation

The first step of the measurement is the incubation phase. Here, the NT-proBNP will bind to the bead. During this step, some clusters are already formed. The process of cluster formation, as described above, is a probabilistic effect, purely based on the diffusion of the components in the solution. This would result in long measurement times, since diffusion is typically a slow process. However, the superparamagnetic properties of the beads can help to accelerate the incubation process. When an external magnetic field is applied, the beads tend to follow this field. Therefore, we will use magnetic actuation using a pulsating magnetic field in order to enhance cluster formation.

Now that the clusters are formed, the actual measurement can be performed. Once again, we will use magnetic stimulation to gain information about the clusters. The number of clusters is related to the amount of NT-proBNP in the sample. A rotating magnetic field (see figure 2) will induce rotational motion of the beads. We will make use of the different shape of clusters in order to identify them. If we illuminate the sample with a laser, the scattered light will be time-dependent, due to the time-varying cross-section of clusters. Single beads have a constant cross-section, and will therefore not contribute to the time-dependency of the signal. Fourier analysis will be used in order to obtain the amplitude of the signal at the cluster rotation frequency. From this, the corresponding NT-proBNP concentration can be found.

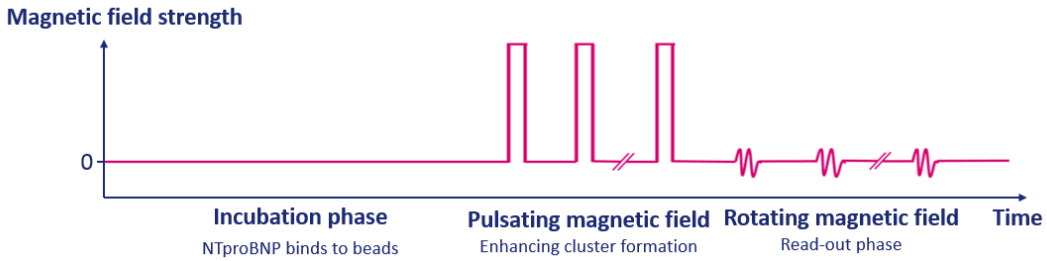

Figure 2: Magnetic stimulation in the cluster assay. First the incubation phase, in which the NT-proBNP binds to the beads. Secondly, the detection phase, in which clusters are formed by a pulsating magnetic field, and the concentration of NT-proBNP is detected by a rotating field.

1.2 Functionalization of the beads

In order for the beads to bind to NT-proBNP, the beads must be functionalized with antibodies. Antibodies are bound to streptavidin-coated beads using Biotin - Poly Ethylene Glycol-N-hydroxysuccinimide (bPEG-NHS) linkers. One end of the PEG-linker contains a carboxylic acid-NHS moiety, which can be covalently bound to an amine goup of an antibody by replacing the NHS group. Each antibody can contain several PEG-linkers. The other end of the PEG-linker contains a biotin-group, which binds to streptavidin on the beads. To prevent non-specific binding, unbound streptavidin is coated with PEG-linkers. Next to that, a second layer of PEG-linkers is applied to shell the antibodies using extra streptavidin-biotin coupling to the additional PEG-linkers bound to the antibodies.

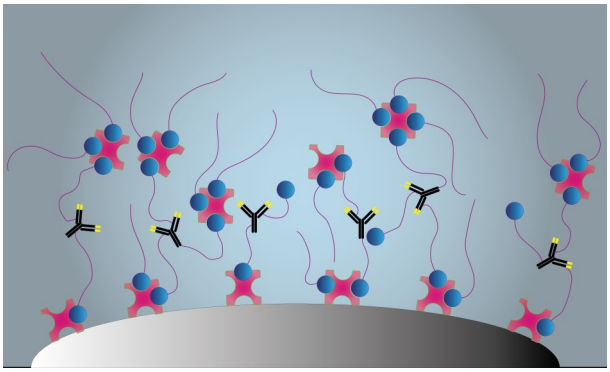

Figure 3: Multilayer bead surface architecture

1.3 Proposed biosensor system

In our biosensor system of our first prototype, we will apply the magnetic cluster assay, as described above. As can be seen in figure 4, a laser (658 nm, 70 mW) is used to illuminate the sample. This causes scattering, which can be measured by a photodetector, located at 16 degrees with respect to the transmission axis. This turned out to be the optimal angle for measuring scattered light from clusters, in our case.

The quadrupole, consisting of four coils which can be driven independently, will provide the external magnetic fields needed for magnetic actuation and for the actual measurement. An Arduino DUE will look after the control of the quadrupole, and a second Arduino DUE is used to transfer the signal of the photodetector to a laptop, which analyses the data.

All of these techniques happen in the 'black box' of the system. For the patient, the use of the device has to be as simple as possible. In order to facilitate the use of the system, a cartridge* has been designed, which is shown in figure 5. The blood sample of the patient will flow in the cartridge, fully based on capillary action. An oxygen-plasma makes the surface hydrophilic to facilitate the capillary action.

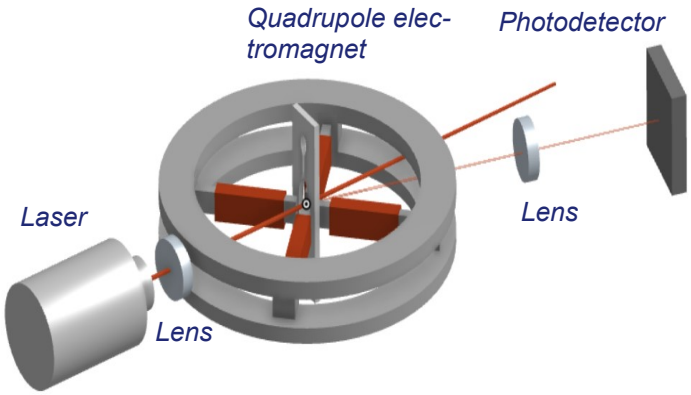

Figure 4: Used setup for the magnetic cluster assay

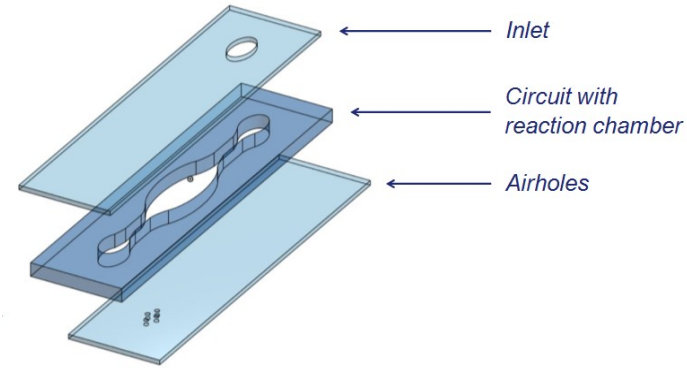

Figure 5: Cartridge design

The beads will be dried in a sugar-solution in the middle of the cartridge, which allows them to solve in the sample as soon as it arrives in the chamber. In the measurement capsule, the measurement will be performed. These cartridges are inexpensive and easy-to-use, allowing for convenient measurements.

2. ANALYTICAL PERFORMANCE

To evaluate the working of our biosensor system, we analyzed the accuracy and reliability of our device, which can be displayed in dose-responses curves (DRC's). This also involves the used sample volume, sample handing and other measurement characteristics. The default settings are listed in the table in appendix I. These settings are used in all experiments, unless stated otherwise.

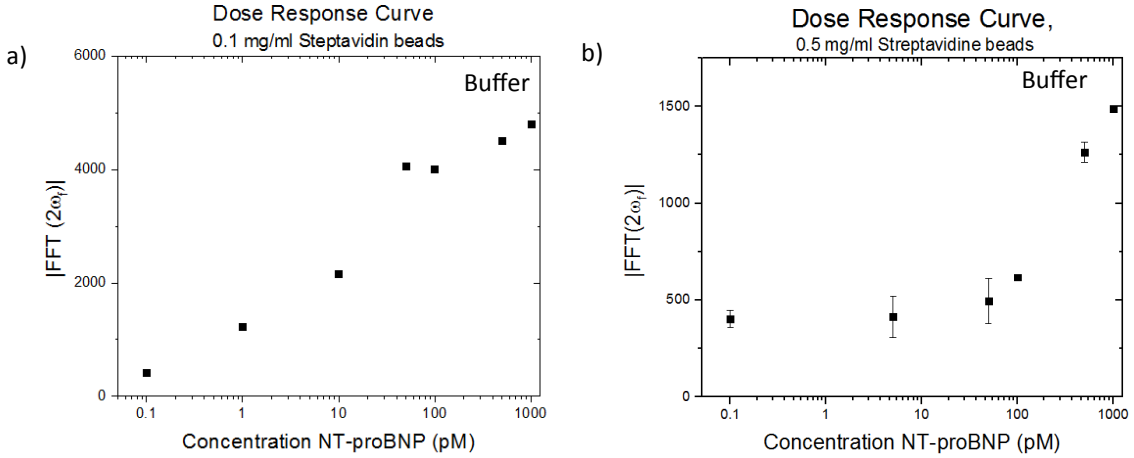

Figure 6: a) Dose Response Curve in buffer with 0.1 mg/ml bead concentration. The assay is most sensitive to the concentration range from 0 to 100 pM. b) Dose Response Curve in buffer with 0.5 mg/ml bead concentration. With this bead concentration, the assay is most sensitive in the range from 100 to 1000 pM.

2.1 Buffer: tweaking dynamic range with bead concentrations

In figure 6 two dose-response curves are shown, both measured in buffer. The curves show the reaction of the assay in presence of NT-proBNP. The bead concentration is determines in which concentration range the assay is most sensitive. The absolute signal, the values on the y-axis are not comparable due to batch-batch chemical variations. However, both batches show a response: the upper asymptote of the signal is in both cases more than a factor three times higher than the lowest concentration NT-proBNP measured. In practice, this means that every new batch of beads developed will have to be tested and the calibration information should be incorporated in the cartridge.

Both curves show a steady signal in their extremities: the DRC with 0.1 mg/ml does reaches an almost steady value for concentrations higher than 100 pM, which is very convincing for a biosensor. If a measured concentration is outside of the dynamic range, the sensor can simply show that the concentration is higher than the highest measurable concentration. In the DRC with 0.5 mg/ml the signal value is stable before the 100 pM, hence here the biosensor could simply show that the concentration in a measured sample is lower than the lowest measurable concentration.

The fact that the dynamic range can be tweaked is helpful in developing other cartridges, for biomarkers which have a different clinically relevant concentration range.

2.2 Plasma: dilution and surfactants

Besides our self-made biosensor, we have used a laboratory set-up* to test our assay in blood plasma. Figure 7 shows the DRC of our assay in 10% blood plasma with extra surfactant added to our bead concentration (Pluronic) and a doubled magnetic field strength during the actuation phase. Although the measurement values are closer together than in buffer and we only have a factor 2 times difference in signal values between out lowest and highest NT-proBNP concentration, the reactivity of the system is still there.

Hence, the slope of our DRC in blood plasma in a major point of improvement. Also, we are aiming at increasing the concentration of blood plasma in which we can measure a clear DRC in our next experiments by tweaking the concentration of surfactants and working with filters.

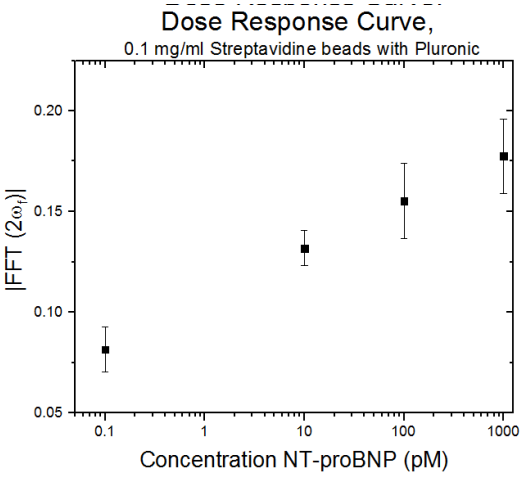

Figure 7: Dose Response Curve in 10% blood plasma with 0.1 mg/ml bead concentration and 50 mT magnetic field

* the main relevant difference between our set-up and the laboratory set-up is the laser power, which is a factor 2 higher in the laboratory set-up.

3. NOVELTY AND CREATIVITY

3.1 Already available

Handheld NT-proBNP testing is already available on the European market, namely the Samsung Lab Geo IB10, the Roche Cobas h232 and the Alere Triage. Besides this, also handheld diagnostics for BNP exist, namely the Philips minicare (upcoming) and the Abbott i-STAT.

The technology used (rotating magnetic cluster assay) was studied by Andrea Ranzoni who published his proof of concept in ACS Nano in 2012 (Ranzoni, Sabatte, IJzendoorn, & Prins, 2012). The technology is patented by the TU/e.

3.2 New developments

Incorporating the rotating magnetic cluster assay in a handheld diagnostic device is new. The results in the ACS Nano Article are retrieved on a large pressure table in the laboratory and hence the set-up had to be miniaturized. To improve user-friendliness we developed a cartridge so that the sample does not need to be inserted into a cuvette. The cartridge also allows the addition of a filter to separate the plasma and hematocrit.

The focus on the patient as user and measuring in a home setting is new for NT-proBNP in Europe. This requires a user-friendly, robust and low cost analyzer and cartridge and good support helping the patients to interpret their measurement results.

We have designed and ordered some prefabricated components like a quadrupole electromagnet and photodetector. We did the assembly, chemical functionalization, production of the cartridge and we wrote the software involved ourselves. We are most proud of the miniaturization of the technique, the extent to which we have defined the use by the patient and the chemical functionalization.

4. TRANSLATION POTENTIAL

4.1 Healthcare application potential

Our biosensor empowers patients and allows them to perform measurements at home. Our sensor targets patients with NYHA classification III and IV, patients with the most severe forms of heart failure. Currently, heart failure patients are being monitored by the cardiology department of hospitals. Every two weeks they receive a call whether any symptoms are experienced. If the health status seems to be deteriorated, the patient is checked with an ECG to determine the ejection fraction of the heart. The blood of the patient can be checked for the concentration NT-proBNP. Weight of the patient can be monitored as well, since this is an important factor in the course of heart failure. The drawn blood is processed in high throughput machines in hospital laboratories. Results are then backlinked in a time between a few hours up to a day. NT-proBNP tests are thus time-consuming and expensive.

Our sensor device reduces logistics and empowers the patients. Nowadays, a heart failure patient spends an average of 3,5 hours per month exclusive travel time on hospital visits, blood withdrawals, etcetera. With the biosensor, the patient will have more control and self-management. It can also give them a confirmation about symptoms they are feeling (Appendix II).

The handling of the device is focussed on patient-friendliness. The patient receives a health kit, containing the sensor device and a blood pressure gauge. The attending cardiologists set up a measurement plan. With a certain frequency, the patient measures the NT-proBNP concentration, their weight and their blood pressure. Blood is collected by fingerstick. A survey among heart failure patients shows that 91,5% of the respondents experiences a fingerstick not as bothersome (Appendix II). The patient places the drop of blood on the cartridge. After a few minutes, the only thing that the patient has to do is to insert the cartridge into the device. The patient then starts the measurement by pushing a button on the touch screen. Using an algorithm, the personal health status of the patient is determined and feedback can be given. The sensor device can communicate with the smartphone of the patient via NFC.

Based on the results of the survey (Appendix II), a combination of options can be shown on the display. The patient chooses once if the device shows the values for each measurement or only the high-risk values by giving a notification. The display always shows the corresponding color. The patient can use an app on his/her smartphone. This app keeps track of the health status of the patient. Furthermore, the patient can choose what the app displays: information on their health status, feedback on their disease and their lifestyle and stay informed about recent researches concerning heart failure.

The measured data will be stored in an online cloud, where the attending cardiologist has access to via an app, which makes it possible to observe the patient from a distance. When the health status of the patient deteriorates, the physician receives a notification. The patient can be called in for examination where after the treatment plan can be changed if necessary.
The anonymous data in the cloud can also be used for research purposes, the pharmaceutical industry, health insurances and policies.

4.2 Industrialization and commercialization potential

The envisioned end product will be a handheld device, not only suitable for the measurements of NT-proBNP, but it can become a multidisciplinary tool in modern patient guidance. The assay technology using antibodies can be manipulated for different biomarkers. Multiple cartridges will be developed after the initial starting-up phase.

In Appendix III our business plan is included. In the research and design phase the product will be optimized and further miniaturized. The cartridge will have increased stability and have an inlet filter. In the implementation phase the sensor device will be tested under many circumstances. Requirements and legislations will be checked in the verification phase. Lastly, the end user will test the device via a trail. Concerning the starting capital, subsidies will be filed.

The selling price of the analyser will be €500,- and of the cartridge €1,-, which will be reimbursed by the Dutch Healthcare insurance system. In Appendix IV a cost analysis of the analyser and the cartridge are given.
The production of the hardware of the analyser will be outsourced to external manufacturers. In the first production round, skilled mechanics are hired to assemble and test the analyser to guarantee it's quality. If we scale up (when also other types of cartridges are launched) and we can afford to set-up a production line for this component, that would allow the price to decrease drastically. Concerning the cartridge, the injection moulding of the plastic elements will be outsourced. The chemical preparation will be kept inhouse.
With the patient as user and a low selling price, our biosensor is unique on the market. Existing (NT-pro)BNP sensors all focus on emergency and hospital care and cost a few thousand euros per analyser and a few tens of euros per cartridge.

Our biosensor can prevent hospitalization and save healthcare costs. Research showed that there are yearly 30.000 hospitalizations due to heart failure and average costs of €15.500 per stay ("Hartfalen - Cijfers & Context, Volksgezondheidenzorg.info", 2017). Around 5000 patients qualify for telemonitoring each year, assuming all of them are elderly and currently only 50% of the elderly makes use of a smartphone or computer (CBS.nl, 2016), this would result in a potential market for our biosensor of around 2500 new consumers per year. In Appendix V an overview of targeted sales for the cartridge and analyser is given.

Currently, there is a patent on the technology underlying the biosensor prototype on the name of the TU/e. This is published on January 5th in 2012 (A. Ranzoni, 2012). T.E.S.T. 2017 could submit a patent on the miniaturization of the technology and the application of the technology in a biosensor. As discussed above, T.E.S.T. 2017 will prepare the chemical parts of the cartridge itself, and thereby keep it a trade secret.

5. TEAM AND SUPPORT

5.1 Contribution of the team members

This year, the TU/e Sensus Team exists of twelve students in which the disciplines of Biomedical Engineering, Applied Physics and Electrical Engineering, in both bachelor and master phase, are represented. This immediately reveals the strength of our team: we have been able to gain new insights into different fields of our biosensor by combining these disciplines. We worked parallel in seven sub teams and helped each other when these disciplines came together. The sub teams and which team members contributed to them will be shown in table 1. The team has come together by their fascination for technology and the motivation to improve health care through this technology.

Subteam	Responsible	Team members
Bead chemistry	Paul Vernooij and Maaike Kraamer	*Joost Bergen, Lotte Hagedoorn, Gaby van Iersel, Amy Lucassen and Sophie Roos*
Hardware	Max Bergkamp	*Jamy van Geemen and Maartje Pontier*
Software	Joost Bergen	*Tim Donders and Imke van der Schoor*
Cartridge	Imke van der Schoor	*Jamy van Geemen and Maartje Pontier*
Translation potential	Maartje Pontier	*Lotte Hagedoorn, Gaby van Iersel, Amy Lucassen and Sophie Roos*
Sponsoring	Amy Lucassen	*Maaike Kraamer and Maartje Pontier*
Public Relations	Lotte Hagedoorn	*Joost Bergen and Tim Donders*

Table 1: task division of the team

5.2 Support

The strength of our diversity can also be found in our wide range of contacts. An overview of the people who gave advice about the technology and people who gave us insights in the market and wishes of all different kind of users will be given in Appendix VI. These people helped in general or in the areas of chemistry, cartridge or translation potential.

5.3 Sponsors

Through the year, T.E.S.T. got five sponsors. A short overview will be given below:

1. Universiteitsfonds Eindhoven: this is a fund from the TU/e that is established to stimulate and support education, research and the student culture at the TU/e.
2. High Tech Campus Eindhoven: at this campus, many technological and medical companies are located. They contributed us financially, but we also gained a lot of knowledge and contacts during the meetings with them.
3. Ademtech: this company is a leading global provider of complete solutions for sample preparation and diagnostic assays. They provide us with different types of superparamagnetic beads, which are an essential part of our biosensor.
4. DSM: this company offers a technical solution in the areas of health, food and materials and help develop products that make life more enjoyable. They contributed our team financially.
5. Johnson and Johnson Medical: they provide most of the products and services of many medical devices. They contributed our team financially as well.

Finally, we were able to get a better financial position in a more creative way, by winning the public price during the TU/e Contest.

6. FINAL REMARKS

We as T.E.S.T. 2017 would like to thank everybody who supported us during the design and development of our biosensor.

6.1 Future prospects

After the competition days on the 8th and 9th of September 2017, T.E.S.T. will recruit new members for the SensUs competition in 2018. We will especially search for students with a fascination for technology and motivation to improve health care. Students will also be selected on the discipline they are studying to form a multidisciplinary team.

As for our project, the design and development of our own biosensor will not stop after the competition days. Our project will continue to completely develop, optimize, verify and validate. Eventually we tend to bring our innovative NT-proBNP biosensor, for the detection and monitoring of heart failure to the market. After this, we will introduce several new cartridges to make our biosensor a multidisciplinary tool to use at home. As we announced: Our biosensor will be your laboratory at home!

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Ranzoni, A., Sabatte, G., Ijzendoorn, L. J. Van, & Prins, M. W. J. (2012). One-Step Homogeneous Magnetic Nanoparticle Immunoassay for Biomarker Detection Directly in Blood Plasma, (4), 3134–3141

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Ranzoni, A., & Prins, M.W.J. (2012). Detection of actuated clusters by scattering., Patent No. US2012003750

APPENDICES

APPENDIX I

Table 2: Default settings measurement

Incubation	
Incubation time	10 min
Actuation	
Total actuation time	130 s
Number of pulses	20
On time	2 s
Off time	4 s
Magnetic field strength	25 mT
Pause between actuation and measurement phase	10 s
Measurement phase	
Total measurement time	2 min
Number of pulses	20
On time	1 s
Off time	5 s
Rotation frequency	5 Hz
Magnetic field strength	3.75 mT

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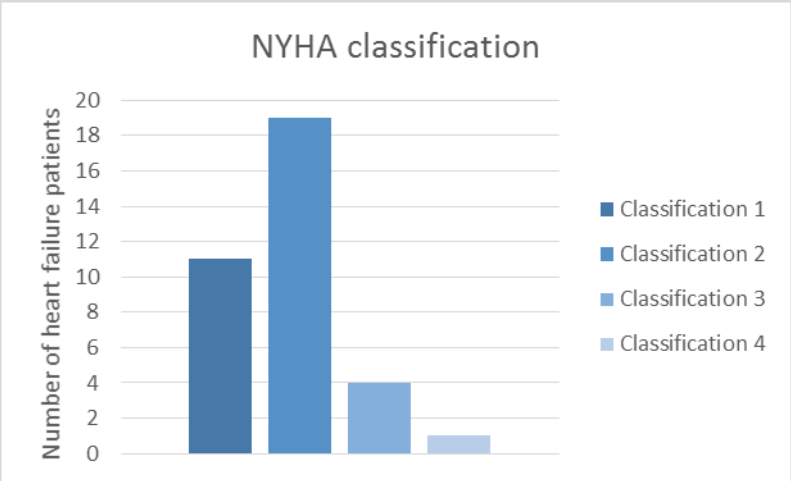
APPENDIX II

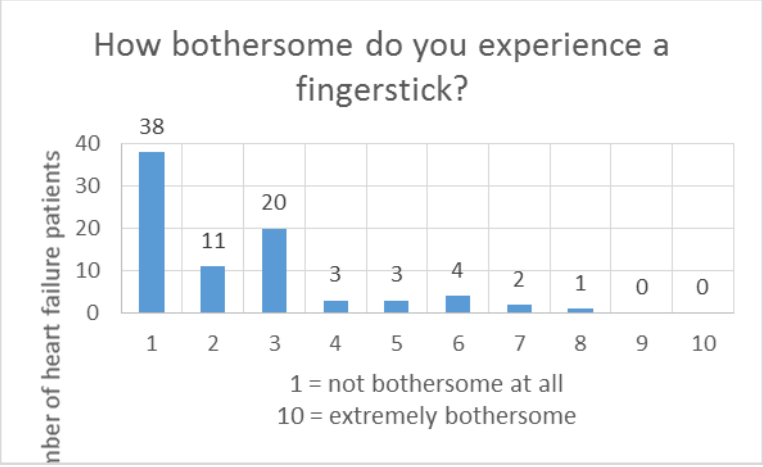
Results from the questionnaires to heart failure patients

23% of the 82 heart failure patients who have participated in an online survey are accompanied in a telemonitoring system via a hospital or a platform. Values they mostly measure are weight, heart beat and blood pressure. Some of them also keep track of their diet and activity. The telemonitoring system is experienced as pleasant, because of the monitoring at home and the direct result. It gives the patients a feeling of safety about their health status. However, the patients indicate that they miss a weekly or monthly report, a confirmation that their disease state is stable.

The target group for the sensor device are heart failure patients who have NYHA classification III and IV. They have the most extreme case of the disease, and monitoring is therefore important. 14% of 36 patients in a follow-up survey have indicated that they have this kind of heart failure. In daily life they experience many complaints: dyspnea, fatigue and tightness. This already takes place during a conversation or when walking up the stairs. This hinders the patients enormously and explains also why these patients are very busy with their health, an average of 3.5 hours per month exclusive travel time.



91,5% of the 82 heart failure patients indicated that they find a fingerstick not bothersome.

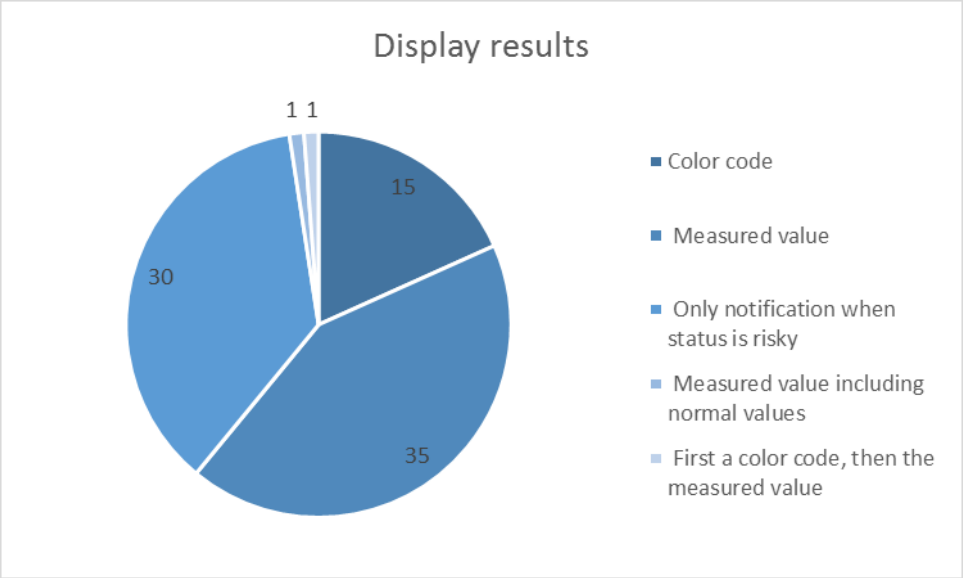


73% is interested to use the sensor device. They would like to have more control about their health. At the moment little monitoring is present, the measurements at the hospital are only a time recording. None of the surveyed patients expects to have any difficulties in the use of the sensor device.

APPENDICES

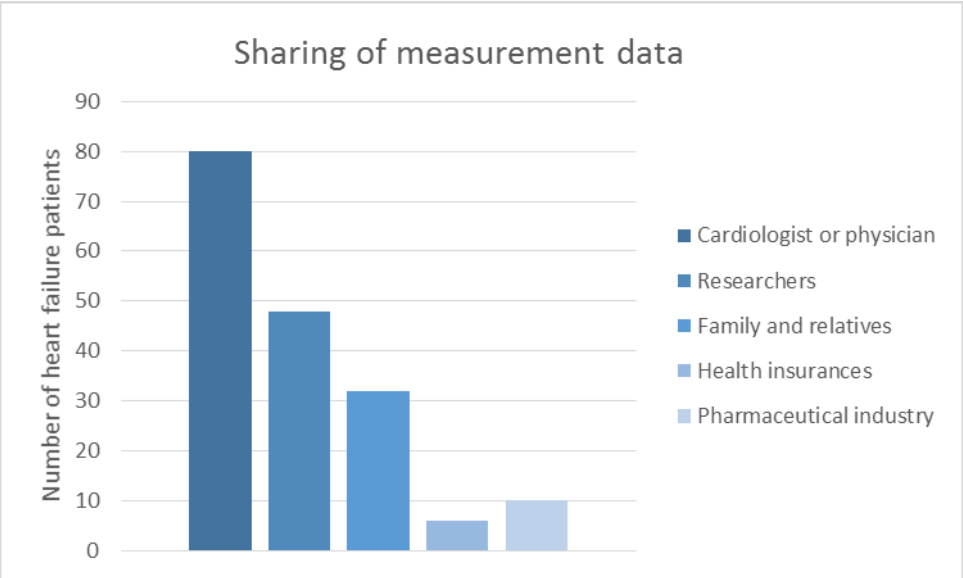
Results from the questionnaires to heart failure patients

Two options are preferred in feedback the patients receive: the display shows the measured NT-proBNP concentration (42,7%) and the display only gives a notification when the health status of the patient has deteriorated (36,6%).



Of these patients, 83% want to receive information about their current health status. About 40% would like to get advice about their disease and their lifestyle. Around 50% would like to stay informed about recent researches concerning heart failure.

From the patients surveyed, 97,6% prefers to share their measurement results with the attending cardiologist or physician. Sharing data anonymously is not experienced as disadvantageous: 58,8% wants to share data with researchers, 12,2% with the pharmaceutical industry and 7,3% with health insurances. Furthermore, 39% of the patients would like to give their family and relatives insight in their health status.



APPENDICES

APPENDIX III: BUSINESS PLAN

RESEARCH AND DESIGN PHASE

For the feasibility of our sensor, the following goals are set:

- **Optimization of measurement parameters** (number of pulses, magnetic field strength, duration of pulses, bead and antibody concentration), so that a dynamic range of 100 to 15.000 pg/mL NT-proBNP is achieved and a standard deviation of ± 25 pg/mL.
  Also, the dose-response curve must saturate and may not decrease above 15.000 pg/mL.
- Further **miniaturization prototype**. The analyser must not be larger than 15x15x7.5 cm, so it will become a handheld device.
- **Increased cartridge stability** (including dried-in beads). The cartridge must be operable for at least six months, which means that the hydrophilic layer and the blocking of the hydrophilic layer must be stable over a long term.
- Addition of a **filter** in the inlet to filter the white and red blood cells from the blood plasma, so that only blood plasma reaches the reaction chamber.
- **Designing production line and protocols for quality control**. Before entering the market a production line allowing larger scale and fully automated production. Also quality control protocols must be developed to ensure the quality of each product per batch.

DESIGN AND IMPLEMENTATION PHASE

After these goals have been reaches, we will explore the limits of our sensor. In this process we will achieve an improved prototype that works in all sorts of temperatures and is able to measure in all sorts of blood, even from patients who receive a lot of medication and whose blood is 'contaminated' with all sorts of substances which are not found in the blood of a healthy person.

VERIFICATION PHASE

Once we have developed the product, we will continue with the verification phase in which we will check whether our product confirms all legislations and requirements we have set in the beginning. The product will be applied for a CE certification.

END USER PHASE

Then our product will be tested in a setting by the end user, via a clinical trial. In our case, this means a trial with severe heart failure patients under supervision of the cardiologist or the heart failure division of a hospital. In this step we will optimize the following specifications:

- Increased user-friendliness of the design and user support. The device must have an intuitive design so that the user must be able to properly use the cartridge and analyser without reading an extensive manual.
- Further integration into existing patient data infrastructure. The home-test results must be stored properly and must be ready for interpretation for all persons involved (patients, cardiologists and other medical professionals). This will be achieved by conforming the sensor to the HL7 standard.

Since we are not trained entrepreneurs, the steps from prototype to market will be taken in cooperation with an incubator, for example Brightlands, which holds close ties to the Maastricht Health Campus.

After full development of our biosensor, we will target the Dutch market,which grows with 40.000 new heart failure patients each year.

Business plan is set up with information gathered in conversations with Philips Healthcare, cardiologists, clinical chemists and the heart failure department of the Catherina Hospital in Eindhoven.

APPENDICES

APPENDIX IV: COST ANALYSIS

PRODUCTION COSTS: ANALYZER

Please note that especially the costs of the quadrupole magnet is based on a very rough estimation, since this component is not commercially available and would have to be custom-made.

Component	Price	Example of a manufacturer
Quadrupole electromagnet	€ 100,-	Sekels GMBH (Germany)
Biconvex lens	€ 5 ,-	WorldHawk (China)
Plano-convex lens	€ 5,-	WorldHawk (China)
Laser Diode + collimator	€ 15,-	Laserland (China)
Photodetector	€ 5	Wuhan Shengshi Optical Tech-
Electronic circuit including amplifiers and NFC tag	€ 25,-	AllPCB (China)
Battery	€ 10,-	Shenzhen Honghaosheng
Casing (injection moulding)	€ 5,-	Shanghai Zetuo Industry Co.,
Labour costs, assembly	€10,-/€ 50,-	Automized/Skilled mechanic
Total	€180,-/€220,-	

PRODUCTION COSTS: CARTRIDGE

In the cost analysis several components have been taken together in the category 'other'. This includes amongst others the plastic, Ademtech Beads, and packaging. When bought in large quantities, these costs are almost negligible in comparison to the costs of the antibodies and blocking agents.

Component	Price per 1000 cartridges	Example of manufacturer
Antibodies	€ 200,-	Abcam
Blocking agent (steric hindrance)	€ 10,-	Nanocs/Sigma Aldrich
Hydrophilization	€ 10,-	Susos
Other	€ 5,-	
Functionalisation process	€ 100,-/€400,-	Automated/lab employee
Total	€ 325,-/€625.-	

MARKET PRICE

Obeying the advice of Philips Healthcare, the production costs are **tripled** to give the market price. For the initial sales there is not enough start capital to invest in a complete automated production line, but in later rounds as the demands increases, this will be possible. For consistence and to conquer a market share we will use one price however, based on the later production rounds. Hence the final selling price of the analyser will be **€ 500,-** and the selling price of the cartridge will be **€ 1,-.**

APPENDICES

APPENDIX V: OVERVIEW OF TARGETED SALES

Based on the expected market, a calculation for future sales is done. Below an overview of the targeted sales for the cartridges (100 cartridges per year per patient) and analyser is given, corrected for the mortality (assumed to be 30% per year) and recycling of the analysers:

Year 1	Amount	Costs (€)	Selling Price (€)	Profit (€)
Analyser	250	220	500	70.000
Cartridge	25.000	0.63	1	9250
Total				79.250

Year 2	Amount	Costs (€)	Selling Price (€)	Profit (€)
Analyser	2500	170	500	825.000
Cartridge	267.500	0.33	1	179.225
Total				1.004.225

Year 3	Amount	Costs (€)	Selling Price (€)	Profit (€)
Analyser	2500	170	500	825.000
Cartridge	437.250	0.33	1	292.958
Total				1.117.958

Year 3	Amount	Costs (€)	Selling Price (€)	Profit (€)
Analyser	2500	170	500	825.000
Cartridge	556.075	0.33	1	372.570
Total				1.197.570

Year 3	Amount	Costs (€)	Selling Price (€)	Profit (€)
Analyser	2500	170	500	825.000
Cartridge	639.253	0.33	1	428.300
Total				1.253.300

In this analysis, the outlook of developing different cartridges in not taken into account.

Targeted sales is set up with information gathered in conversations with Hartstichting and Philips Healthcare and is based on the expected market.

APPENDICES

APPENDIX VI: TEAM SUPPORT

Table: Support given to T.E.S.T. 2017 by different people from different areas

General	Chemistry	Cartridge	Translation Potential
Dr. L. J. van IJzendoorn and M.R.W. Scheepers.	Max Scheepers (PhD, group of M. Prins), Jan Vinkenborg (Philips), Marco Hefti (Future diagnostics), Andrea Ranzoni, Sam van Dun (PhD, group of Prof. Brunsveld).	Dr. J. Maas and Ir. A. M. de Jong.	Cardiologists: Dr. Ramón van de Ven (St. Anna Hospital), Dr. Harold Kemps (Maxima Medical Centrum) General practitioners: Dr. Joep Gondrie and Martine Dam. Nurse practitioner cardiology: Cindy Verstappen (Catherina Hospital) Clinical chemists: Saskia van Loon and Lieke Klinkenberg from Catherina Hospital. Industrial experts: Joost Maas & Jeroen Nieuwenhuis (both Philips) Patient organizations: 'Hart & Vaat groep' and 'Hartpatiënten Nederland'. Insurance companies: National Health Care Institute and healthcare insurance Coöperatie VGZ. Health Platforms: Margit Ruis (Mijn Gezondheids Platform), Arnaud Parise (iHealth Labs Europe), Dr. gor Tulevski & Sebastioaan Blok (both HartWacht)
They gave us a lot of support. During the entire project we discussed our ideas and results with them.	Jan, Marco and Sam gave us tips to understand the carboxyl and streptavidin protocols. Max was our day-to-day focal point for our questions.	Both gave us advice about the materials and basic principles of a cartridge.	Because we have been able to contact all this people, we have had the possibility to gather the ideas of everyone who could be involved by the realization of our biosensor.