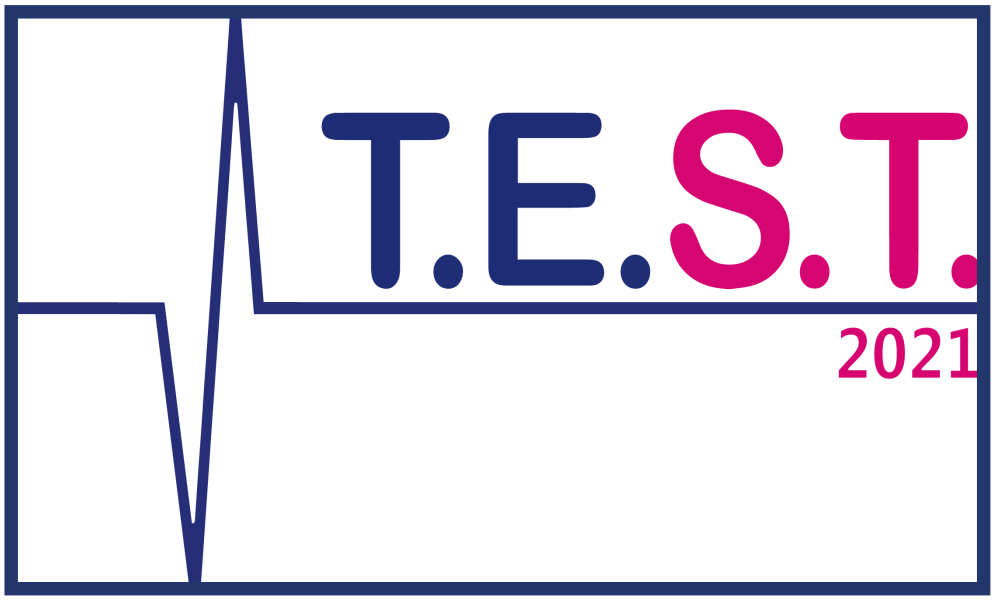
Team Results Document

T.E.S.T.



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SensUs

TU/e EINDHOVEN UNIVERSITY OF TECHNOLOGY

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1. Summary for the SensUs website

T.E.S.T presents an innovative particle-based biosensor to measure influenza A in saliva. In an immuno-assay sandwich with particle labels, high-affinity antibodies are used to measure the HA1 protein of influenza A with high specificity. This biosensor technology offers a single-particle resolution, where the exact number of observed particles reveals the HA1 protein concentration and thus the severity of the infection [1]. T.E.S.T developed a fully functional prototype, using automated software to count the observed particles. Furthermore, the simplicity of the sensing principle allows for easy interchanging of different virus strains, so that any virus can be detected. It also offers possibilities for testing a person on multiple influenza strains at the same time. A feasible business model has been developed which includes further development into a miniaturized user friendly sensor.

2. Biosensor System and Assay

2.1 Molecular recognition and assay reagent

Preparation
For the detection of Hemagglutinin (HA1), team T.E.S.T. proposed an innovative assay based on a sandwich immuno-assay. The assay principle consists of two major components; an antibody functionalized to the surface and an antibody functionalized onto streptavidin-coated superparamagnetic particles (Dynabeads MyOne Streptavidin C1, Invitrogen), of which both have a high affinity (Influenza HA ELISA pair set, Antibodies Online) against HA1. As visualized in Figure 1, the antibodies on the particle are called detection antibodies (dAb, purple) and the antibodies on the surface are capture antibodies (cAb, green). dABs are functionalized with biotin moieties to attach the dAbs to the particles, therefore enabling streptavidin-biotin coupling between the dAb and the particles. Subsequently, the particles are blocked with mPEG-biotin (PG1-BN-1kk, Nanocs) and BSA to prevent nonspecific binding of the particles to the surface. The glass surface is functionalized with cAbs using physisorption and subsequently blocked by BSA.

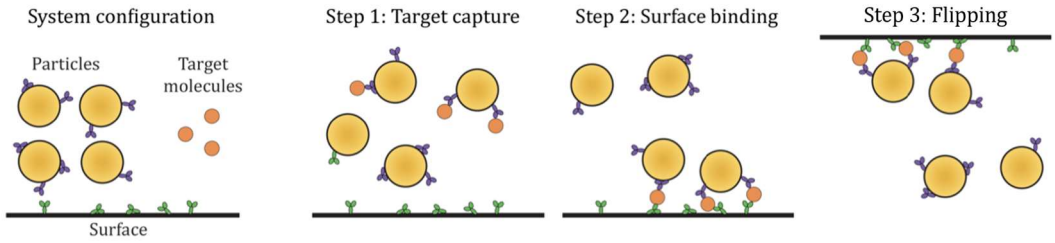


Figure 1. Functionalized particles moving in solution and ultimately diffusing to the surface. Upon finding a target molecule (HA1 in this case), these particles capture the analyte from the solution in the first step of the system using dAb. Binding to an accessible antibody on the surface leads to the formation of the molecular sandwich interaction. At last, the surface is flipped to remove the unbound particles from the surface of interest. Altered From: [2].)

Assay
The sample with HA1 is added to dAb-functionalized particles where the dAb concentration is relatively high compared to the HA1 so that HA1 proteins can rapidly bind to the dAbs, forming a particle-HA1 complex (*Figure 1. step 1)*. After incubation, the mixture is added to a flow cell where particle-HA1 complexes through which these particles can freely diffuse due to Brownian motion, and therefore can bind to the cAb-functionalized surface. When the particles bind a particle-HA1-substrate complex, or so-called molecular sandwich has been formed. (*Figure 1. step 2*).

In case of no binding, the particles will be freely moving through the fluid. These unbound particles are allowed to sediment away from the surface due to gravitational force by turning (flipping) the flow cell upside down after which only the specifically bound particles remain at the substrate surface (*Figure 1. step 3*). [2]

The assay is relatively easy. There are not many steps needed to process the samples and the biochemical reagents used in the assay. The equipment that is used in this assay is also not very expensive[3][4][5][6]. The proposed technique is easily adaptable by replacing the antibodies, for instance with affinity to other strains or other biomarkers. Taken together, the particle imaging technology is chosen to achieve the sensitivity, specificity, precision, and accuracy needed to acquire reliable data valuable in minimizing the impact of Influenza A on people's life.[4] [7]

2.2 Physical transduction

The number of bound particles has been determined by bright field microscopy where only a small magnification is required to observe and count particles with a typical size of 1 µm in diameter.
When using bright field illumination, the sample is directly illuminated with incident light [2]. Objects, such as the particles, scatter and absorb light, which results in dark spots against a bright background. For a high contrast between these dark spots and the bright background, the sample needs to be placed at the working distance from the objective, so that the particles are within the depth of field.
The resulting magnified image can be imaged using a camera for analysis. Where the contrast between the particles and the background is used to detect and count the particles bound to the surface. The number of observed particles scales directly with the HA1 concentration.

2.3 Cartridge Technology

The provided cartridges comprises functionalized glass coverslips (Rogo Sampaic Glass Microscope Slides) with a fluid cells sticker (Grace Biolabs) attached to the glass surface by an adhesive layer, resulting in a flow cell with a volume of 23 µL (see Figure 2). The shape and size of the flow cell can be found in figure 2. The sample needs to be pipetted in the inlet and excess fluid needs to be wiped off at the outlet with a simple tissue. After the sample is added, the inlet and outlet are covered with tape. The cartridge is now ready to be put in the biosensor with the Fluid Cell facing up. After incubation, the cartridge is flipped.

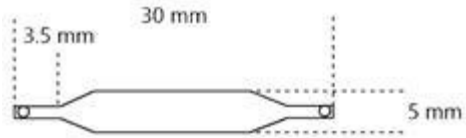


Figure 2. The shape and corresponding measurements of the used flow cell. Showing one fluid chamber, an inlet and an outlet.

2.4 Reader instrument and user interaction

Reader instrument

The reader instrument roughly consists of five components. All components are mounted on a back plate. First the camera (Blackfly S USB3, Flir) is attached to a translation stage (ThorLabs) to the backboard. This translation stage enables focusing of the camera. Beneath that, an objective (10x/0.25 DIN Achromatic Commercial Grade, Edmund Optics) is kept in place by an objective holder and a cartridge holder is attached. At the bottom a green LED light (ThorLabs) is kept in place by a LED mound.

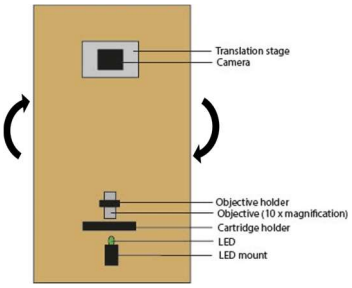


Figure 4. *Vlaflip* design. Showing set-up components and flipping direction

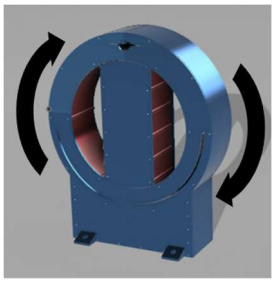


Figure 3. *Donut* design. Showing the casing and flipping direction

As stated before, the cartridge needs to be flipped. For this requirement two prototypes have been developed. The *'Vlaflip'* (*Figure 3*) set-up will be able to be completely flipped, which makes sure everything will stay in focus and aligned. The next set-up, the *'Donut'* (*Figure 4*), is designed to make the flipping step user friendly. In this design the sample is simply flipped by turning the knob on the back.

Size and design of the instrument

The size of the *Vlaflip* is 150 x 150 x 500 mm. The design is not made aesthetically pleasing to enable easy access for adjustments. The *Donut* however is designed to be produced for users. This device currently has dimensions of 406 x 105 x 475 mm and can be reduced further in size. All components of the Donut are closed off, making it impossible for the user to accidentally interfere with components and making the design more sturdy.

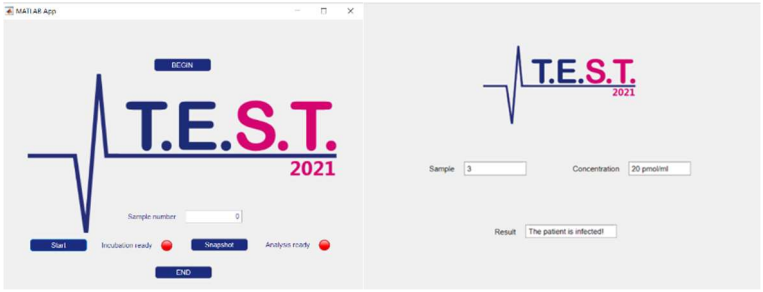


Figure 5. a. Graphical user interface. b. Result pop-up, showing the measured concentration

User interface & user-friendliness

A graphical user interface (*Figure 5a*) is designed to improve the usability. The app can be installed on a computer, to be used together with the biosensor. The app will activate the software and indicate when proceeding to the next measuring step by giving green lights. The user is guided by the app when performing a measurement, ensuring a feasible image is acquired. A threshold of the intensity is used to separate the particles from the background, after which the total amount of particles present in the field-of-view is determined, all using Matlab software. Since the number of observed particles directly scales with the HA1 protein concentration, the input HA1 concentration can be determined and visualized by exportation of the data to an Excel file or a pop-up screen (*Figure 5b)*.

Performing a measurement

Several handling steps are required to perform a measurement: **Step 1**: When starting the first measurement, click "BEGIN". **Step 2**: Add the saliva sample into the particle solution and wait for the incubation. **Step 3**: Pipet this mixture in the flow cell and press "Start" on the app. **Step 4**: Slide the flow cell into the cartridge. **Step 5**: Wait until the light turns green and flip the sample. When using the *Vlaflip,* this can be done by flipping the entire biosensor. When using the *Donut*, the knob needs to be turned. **Step 6**: Press "Snapshot" on the app. **Step 7**: Wait until the light of analysis turns green. **Step 8**: When performing a new measurement, go back to step 2. When the last sample is measured, press "END" to get results from all the performed measurements.

3. Technological Feasibility

3.1 Calculations of ratio particles vs HA1

Calculations were made for the maximum amount of antibodies per particle to give an indication of the concentrations of antibodies that should be used to functionalize the particles in the lab. In addition to this, simulations have been performed to estimate the ratio between cAb-functionalized particles and HA1, which would result in a sufficient signal. This ratio was used as a starting point for designing the assay in the lab.

The calculation of the maximum amount of antibodies on a particle was done using the typical binding capacity of the particle for a biotinylated IgG antibody. The following assumptions were made for these calculations; a particle is perfectly round and has the same density as polystyreen. Our calculations showed that each particle could maximally have 4.4 x 10^4 cABs on its surface after functionalization (for elaborated calculations see Appendix A0.1). This amount is used in the simulations and to calculate the optimal incubation concentration of biotinylated antibodies with particles (See appendix A0.2).

Subsequently, simulations were done varying the number of particles, HA1 concentration, geometry of the flow cell and the field of view. A Poisson distribution was used to correlate the number of particles that were bound in the FOV to the total concentration of HA1 in the reaction chamber.
The amount of HA1 proteins needed to be much smaller than the amount of antibodies, resulting in the assumption that all HA1 proteins would be captured from the solution. Another assumption made was that every particle that binds to a protein also binds to the surface.
The simulations showed a 1:1 ratio between HA1 and particles with precision roughly equal to or lower than 10% in the required concentration range. A field of view of 3*0.889e-6 was found to be sufficient and in line with the specifications of the camera.

Varying the particle concentration to a working concentration of 10 µg/mL shifted the limit of quantification of the assay for concentrations below 6000 pg/mL By varying the particle concentration we can regulate the dynamic concentration range of HA1.

3.2 Blocking

The designed assay relies on the specific interaction between cABs on the particles, HA1 and dABs on the surface. The amount of nonspecific interactions between particles and surface was quantified using a negative control where no HA1 was added. In the early stage experiments it was noticed that no significant differences were measured in the counted number of particles in the blank and in an assay using 10,000 pg/mL (the highest concentration required for the Sensus Competition 2021), due to the amount of non-specific binding. Hence, BSA blocking of both the particles and the surface was added to the protocol in order to bring the background signal down. In the future, the background signal could be lowered even more by using blocking agents on the particles and surface that have a repelling force e.g. ssDNA strands.
Secondly, it was observed that particles are prone to clustering. Particle clustering has a negative effect on the automated counting process of particles because these led to more false positives. Particle clusters have a higher tendency to bind via nonspecific interactions on the surface. Applying ultrasonic vibrations before the incubation phase to the particles resulted in a decrease of cluster formation. To further diminish clustering, the particles were blocked with PEG-biotin complexes. Hence, PEG-biotin blocking became an essential step in the particle functionalization protocol.

3.3 Biosensor performance

Dose-response curve
The assay was tested by performing measurements on the *Vlaflip* biosensor. Six different HA1 concentrations were tested; 50.000, 6.000, 85, 10 and 0 pg/mL. The used particle concentration is 10 µg/mL, combined with the different HA1 samples in a volume ratio of 1:1. The corresponding number of particles were then plotted against the HA1 concentration, resulting in a dose-response curve (Figure 6). The graph shows an increase in response at 50.000 pg/mL, meaning the sensor was only sensitive in the upper part of the relevant concentration regime. In an attempt to shift this increase towards the lower HA1 concentrations, the concentration of particles was lowered to 1 µg/mL in a follow up experiment. Lowering this concentration will result in a higher ratio of HA1 against the particles, resulting in a bigger chance of binding between the particles and the HA1. Therefore lower concentrations will show more bound particles which results in a lower limit of quantification.

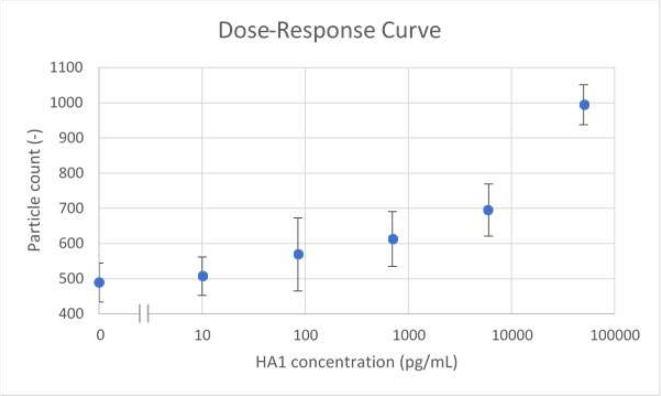


Figure 6. Dose-response curve of six different HA1 concentrations. Average values of 30 pictures are plotted together with corresponding standard deviation.

For these measurements, the HA1 sample and particle solution were incubated for 5 minutes. The same time was taken for the incubation of the flow cell with this mixture. Resulting in a total measuring time of about 15 minutes per sample, which can be optimized by tweaking both the particle concentration and the incubation time accordingly. When the number of particles is decreased, the time needed to capture all the HA1 proteins from the solution will increase. This equilibrium time will be above 600 second for different Kd's, when using a 0.01 mg/mL particle solution. More particles could be used to decrease this equilibrium time, taking into account that this could lead to more non-specific interaction (See Appendix A0.3). More experiments have to be performed to indicate the ideal particle concentration resulting in a suitable measuring time, as well as an adequate sensitivity.

Limit of quantification

To know what the lowest concentration is that can be measured with a precision lower than 10%, the limit of quantification was calculated. The calculation was based on the previous dose-response curve. 30 pictures were taken of every concentration, in order to maintain calculated precision, of which a standard deviation of the counted particles per concentration was determined. The particles were added to the HA1 sample in a ratio of 1:9 while using a particle concentration of 10 µg/mL. The ratio of the standard deviation to the mean was plotted for each concentration, resulting in figure 7. A fitted line was applied from which the intersection of the 10% line could be defined, giving a limit of quantification of 1.8e4 pg/mL.

This sensitivity is predicted to be improved by taking a larger field of view (See Appendix A0.4), which can be achieved by using a camera with a larger sensor. When choosing a sensor size, it should be taken into account that a larger sensor needs better resolution. Each particle needs to be represented, this enables the resulting picture to be analyzed.

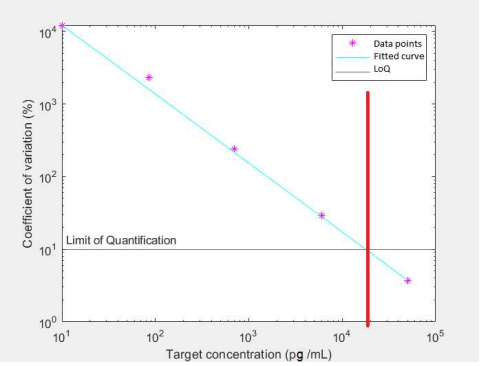


Figure 7. Calculation of the limit of quantification for our biosensor using our software. The red vertical line shows at which HA1 concentration the coefficient of variation reaches 10%.

3.5 Improvements on cartridge and reader instrument

Furthermore, the cartridge and sample handling could be improved. The expiration rate of the cartridge can be elongated by using sugar preservation on the surface, which makes sure the cartridge gives the same performance for a longer time (See Appendix A0.5).

The sample handling requires pipetting for which trained personnel is needed. To make the biosensor more user-friendly, the pipetting process can be replaced by a process similar to that of the current at-home SARS-CoV-2 rapid antigen tests (Figure 8). A saliva sample can be taken with a swab and this swab is to be inserted in an extraction tube, containing a buffer solution. By turning the swab around in the tube, the saliva mixes with the buffer solution. Then, using the extraction tube, a few drops of the solution can be deposited on the flow cell. The flow cell contains particles which for instance are dried with sugar preservation. This is also possible for non-trained people and therefore suitable for at-home use.

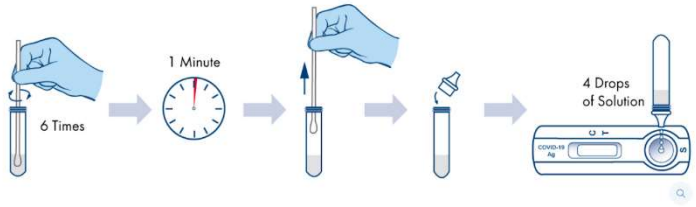


Figure 8. Sample hadeling of the at-home SARS-CoV-2 rapid antigen test [8]

Lastly, in the current *Donut* design (Figure 3) the sample is flipped manually, by moving a knob from right to left through the half-moon shaped slit. In the future, this could be automated by gears and a motor, making this step more user-friendly. In addition, it will be possible to downsize this device following additional research. The microscopic setup can be downsized using smaller digital microscopes, which would reduce the size of the *Donut* to about 100 x 50 x 100 mm.

4. Originality

4.1 Team Captain

The team started with the development of a biosensor, inspired by the continuous-monitoring BPM technique, which was originally developed by Menno Prins [2]. Low-affinity binders however were not widely available so together with the supervisors the choice was made towards end-point measurements, which enabled using high affinity binders. This choice resulted in multiple changes which led to a more simple assay and detection. In BPM a video is made and analyzed to see the change in motion between a bound and unbound particle. However, this interest shifted when the choice for end-point measurements was made, making only the bound particles important. Instead of a video, the team decided to just take a picture. To make sure only the bound particles are visible in the picture, the supervisors advised us to separate the freely moving particles from the bound particles by either magnetic forces or gravitation. The team decided to flip the sample, making the unbound particles sediment away from the surface of interest. To implement this flip into the biosensor, the team thought of several ways to do this, resulting in two prototypes; the *'Vlaflip'* and *'Donut'*.

Different parts of the biosensor, like the camera and translation stage, were ordered. Other parts were designed by the team itself. This includes the objective, cartridge and LED holder, as well as the entire housing of the '*Donut'*. Drawings for these objects were made, 3D-printed and optimized multiple times.

For the used assay, first calculated concentrations of antibodies and particles were used. Afterwards these concentrations were adapted by the team according to the results which were received in the lab. Blocking with BSA and PEG-biotin was also added on advice of our supervisors to prevent clustering.

4.2 Team supervisor

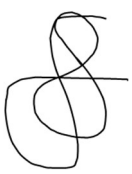
Sensing principle The Team started with a broad literature search on assay and sensor principles in order to get a feeling for the requirements to design and build a sensor for the detection of influenza viruses. They quickly realized that support from a local research group would be very helpful and came up with a short list which included the Biosensing by Particle Mobility (BPM) technology developed in the group of Menno Prins. This technique, primarily developed for continuous monitoring, involves the detection of particle association and dissociation from surfaces by studying particle mobility in time. The team found out that the required low-affinity antibodies are not available for the HA1 protein but did realize that particle binding was easy to detect using microscopy hereby drifting away from the BPM concept to an end-point measurement in a sandwich assay with particles as the label. By applying this concept they could take advantage of the practical knowledge of particle preparation and detection available in the Prins' group and simultaneously use their own conceptual idea of the assay.

Feasibility After discussions with the supervisors on the required sensitivity and involved timescales, the team independently carried out calculations to estimate the required particle concentrations and designed a system which uses gravity to discriminate bound from unbound particles. Besides scanning assay parameters such as particle concentrations and surface loading, additional experiments have been carried out with blocking agents to prevent nonspecific particle binding.

Detector design The mechanical design of the detection system had been carried out completely independent from the supervisors using 3D printing technology. The supervisors only asked critical questions on the integration and adjustment of the optical components but the associated challenges are completely solved by the team independently.

dr. L.J. van Ijzendoorn
(supervisor team T.E.S.T.)

R.M. Lubken
(laboratory supervisor team T.E.S.T.)



S.D.H. ten Damme
(team captain team T.E.S.T.)

A.P.M. Baeten
(team vice-captain T.E.S.T.)

5. Translational potential

5.1 Business model canvas

Figure 9. Business Model Canvas

5.2 Market description

Key factors pushing the point-of-care biosensor market are identified as being the increasingly aging population, the significant global demand for transmission pattern monitoring solutions, and the pressure to find biosensing solutions to improve case management recommendations leading to more efficient allocation of health resources.[9][10]

More concretely, the global influenza diagnostics market grows with a compound annual growth rate (CAGR) of 6.9 percent, expected to continue for the period of 2021-2026. Within the total market size of 420 million USD in 2018, a valuation of approximately 320 million USD is reserved for FDA-approved Rapid Influenza Diagnostic Testing (RIDT) products. Assuming the RIDT market will grow with equal CAGR, the RIDT market will be 510 million USD in 2025. To enter this market segment, T.E.S.T.'s biosensor needs to abide by FDA's rule for approval with a hard threshold of 80% sensitivity, in this case defined as the number of true positives (TP), divided by the number of true positives plus false negatives (FN).[11] [12]

To illustrate the interactions between T.E.S.T.'s biosensor and its users, a customer journey is made to visualize the care pathway as a point-of-care monitoring aid for the high-risk population, seen in Appendix A1.1. Additionally, in Appendix A1.2, the care pathway is provided to establish the place of T.E.S.T.'s biosensor to monitor transmissions at large events. Taken together, these two customer journeys show the true versatility of T.E.S.T.'s biosensor, increasing its value to minimize the impact of future pandemics.

5.3 Stakeholder desirability

Each year, on a global scale, an estimated 1 billion cases result in 3-5 million severe cases of influenza, and 290.000-650.000 deaths are related to influenza [13].

In addition, the World Health Organization reflected on the effectiveness of a pandemic response whilst in the risk assessment phase. Various studies (seen in Appendix A2) suggest that hospital quarantine, vaccination, and the use of antiviral stockpiles are highly cost-effective manners of pandemic responses. This even accounts for mild pandemics. These measures are indicated with the green circles. [14] T.E.S.T. believes that following the current Covid-19 pandemic, early diagnosis will be viewed as a potential cost effective measure to stimulate voluntary quarantine and social distancing of which the efficacy has been demonstrated. This in turn, would increase the demand for biosensing innovations done by T.E.S.T. in future pandemic preparedness protocols.

Naturally, such a demanding challenge attracts certain parties competing to gain market share. To assess T.E.S.T.'s particle imaging based biosensor's competitive position, competitors have been evaluated. Particle imaging has the potential to be applied for a wide range of affinity molecules, making it an attractive biosensing principle to expand on. Because the principle is so simple, the strain or strains that are to be detected can easily be adapted. The imaging principle behind the particle based technology is additionally advantageous over most of the other RIDT solutions, since imaging using a surface with particles on it, can easily be scaled up. The

simplicity of the biochemical assay allows for bulk production due to minimal steps and reactants. Compared to alternatives, this offers cost-effective biosensing.

The core products and services of team T.E.S.T. consist of a point-of-care sensor based on particle imaging, which is rapid, affordable, and easy-to-use for HA1 from Influenza A in saliva. It should aid in case management recommendations for the high-risk population, as well as aid in global transmission pattern monitoring (e.g., at big events, airports, etc.). To achieve this, further steps in clinical studies need to be done to become FDA-approved, complimented with cost-effective analyses to reinforce the competitive positioning.

The essence of the value proposition behind the offered core products and services can be seen as an on-going challenge to improve this team's solutions to optimize the product-market fit. To do so, value- and customer profile maps are used illustrating the pains and gains, the prior without T.E.S.T.'s sensor and the latter with this team's biosensor respectively. See Appendix A3 for the pains and gains.

The framework for decisions moving forward is based on validating the sensor's use-case for the intended customers to optimize for product-market fit.

5.4 Business feasibility

In the short-term, at most until 2024, continuous research and development at the Eindhoven University of Technology (TU/e) is needed to improve the biosensor. Assays need to be done to further optimize the limit of quantification and also the sensitivity (TP/(TP+FN)) of the set-up, and to improve on the signal-to-noise ratio (by reducing the signal of the blank, e.g. by reducing clustering). In parallel, team members need to be "in the field" to have more in depth conversations with the stakeholders to optimize the product-market fit. The T.E.S.T. team is confident in the particle imaging potential to be an attractive platform to expand to different strains, combatting future influenza strains among other interesting diseases, viruses and therapeutic monitoring.

Moving forward, a partnership with antibodies-online.com will be formed to keep using the antibodies. Telefyne Flir has sponsored the camera for the current prototypes and a partnership will be win-win for both parties in the future, because it will gain the company brand awareness in the healthcare innovation community. ThermoFisher is a third sponsor which T.E.S.T. would be interested in to agree on a partnership helping in the manufacturing and scaling-up aspect of the business. The Fluid Cells of Grace Biolabs are specifically made, hence becoming partners would benefit T.E.S.T. in offering more consistency of cartridge quality and reducing the price of the cartridge. However, to reduce the costs of cartridge production (which are currently quite high, see Appendix A5.1) and upscale the production, the cartridge could eventually be produced by means of injection molding and the help of new sponsors is needed [17]. Injection molding is also a viable option for reducing the costs and upscaling the production of the larger components in the biosensor. On-going research and development will be the priority of T.E.S.T. to build a long-term thriving customer connected organization. A Swot-analysis, seen in Appendix A4, gives more insight into the biggest resource, being the T.E.S.T. team. Partnering up with experts in academics as well as in the business world will help in finding the necessary product-market fit to enter the Dutch and subsequently American market, the latter being the most attractive.

5.5 Financial viability

Looking at the financial viability of point-of-care biosensing as an aid for antiviral treatment, biosensors exceeding the 68% sensitivity threshold will be cost-effective for epidemic periods. As mentioned earlier, to enter the FDA-approved RIDT market, the sensor's sensitivity needs to exceed the 80% mark. For sensors with a sensitivity of 90% and higher, application will become cost-effective even in non-epidemic periods. Tests with a price point below 46 dollars do have financial viability. On top of that, probabilistic sensitivity analyses showed that the use of a POCT strategy is cost-effective in 2/3 of the use-cases for influenza, with the cost per life year saved fixed at 50.000 USD [18]. To be competitive, computational simulations assist in more logical decision-making regarding health resources and thus make T.E.S.T. 's biosensor even more cost-effective in the future. In doing so, this sensor can help prevent future pandemics.

The POC-RIDT market is a fragmented market, meaning that there are no organizations dominant enough to influence the entirety of a complete market in their direction [19]. On the contrary, more than ten key players are identified in this market (e.g., F. Hoffmann-La Roche AG (Switzerland), Thermo Fisher Scientific (US), and Abbott Laboratories (US)), leading to open competition within the market [11]. This leads to the assumption that for T.E.S.T., less than five percent in global market share is achievable. Hence, the assumption is made that the target market share is 1%, reached at the 5-year market. This is calculated to be 5.1 million USD based on the top-down approach used in section 5.2. Entering this market straight from the start would require more resources than the team currently has available and would increase complexity to find product-market fits for all possible suitors at once. This drives the R&D costs and requires rapid scaling. The costs will therefore become too high to be competitive against the well-established big players. Hence, market penetration in the Dutch market is seen as the initial go-to commercial strategy during the proof-of-concept phase. This can be done by specializing T.E.S.T.'s biosensor for municipal health services to aid for monitoring the influenza season start and end, transmission pattern monitoring among the risky population, and detecting unexpected as well as unusual events (e.g., outbreaks of influenza outside the typical season, severe influenza among healthcare

workers, or clusters of vaccine failures). Agreeing on a licensing deal with the municipal health services will lower marketing costs, increase focus of R&D resource allocation, and will lead to finding more efficient product-market fit. Variable costs (e.g., chemicals needed for the biosensor) can be equally substantial for both approaches taken.

Specific numbers of the market share of RIDTs in a national (in T.E.S.T.'s case the Netherlands) market is not available now. Therefore, assumptions for the national market will be made based on the national GDP compared to the global GDP numbers, being 1 billion USD and 87,55 billion USD respectively [20][21]. Hence, the percentage share of the GDP of the Netherlands accounts for approximately 0.79 percent of the global GDP. Additionally, the assumption is made that a first world country like the Netherlands will experience the same growth that is expected on a global level, explained earlier. Especially, because the T.E.S.T. is based in the Brain Port Region in Eindhoven which is a fertile environment for innovations worldwide. Hence, it is a safe assumption to make.

Additionally, overall complex, and high-tech medical technology accounts for 0.55 percent of European countries based on research of the European commission. Within a GDP of 909 million euro in the Netherlands this would account for roughly 5 million euros. This is in line with the order of assumed market size in the Netherlands based on the global numbers that were given before. So the assumption is grounded [9].

The complete POCT market of 510 million USD would therefore account for a Dutch POCT RIDT market accounting for 4.15 million USD. Based on a smaller market, the Netherlands, the assumption is made that a realistic market share is 1-2 percent per year during the proof-of-concept phase. Therefore, a safe assumption is made that a market share of 5 percent is feasible for differentiation and 10 percent for market penetration after 5 years. Using these assumptions, the following market shares may be viable:

Market penetration: Entering the entire Dutch POCT market of 4.15 million USD with a market share of 10 percent may lead to 400.000 USD in sales after 5 years. This will be used in the first proof-of-concept phase to fund research and development. After this, T.E.S.T. will target the global market, firstly aiming for 1% of the global market (5.1 million USD) and after 10+ years 10% (51 million USD).

The manufacturing of the cartridge costs € 2.89, see appendix A5.1. The total costs of the prototype reader as it is right now € 1234, see appendix A5.2. These prices are without factoring in the sponsorship deals. With the intended partnerships, these prices are estimated to drop to € 0.30 for the cartridge and € 123 for the device. In doing so, the device could be made from aluminum to cut down on production time, or be made by means of injection molding. For payrolling and marketing, 10% from the material price is assumed to be needed. This would bring the price of the device up to € 150. The cost calculations can be found in Appendix A5.

Private healthcare facilities, municipal health services, healthcare insurance companies, organizers of big crowds (e.g., airports, festivals, etc.) buy the device for € 500. Service costs will be on a fixed hourly pay depending on the needed maintenance. The sales price per cartridge will be put at € 7.50, which is prized at a competitive price point. With higher manufacturing, the cost can be brought down. The buying party will be responsible for the further distribution of devices and or tests.

Considering the largest group within the Influenza high-risk population, being elderly people, an estimation is made that in 2030 roughly 4.3 million people in the Netherlands are aged 65 and over, with 22% being classified as high-risk.[22] This amounts to 946.000 people in the high-risk group.
Assuming 20 devices will be sold per year over a five-year period to municipal health services, this will amount to 100 devices and € 50.000. If we want to reach the targeted 400.000 USD (Roughly € 340.000), this would mean still € 290.000 needs to be done in sales to reach the target market share. Dividing this amount by the price per cartridge returns approximately 38.667 tests, or 4% of all high-risk elderly people. This means there is still room for growth in this high-risk group.

6. Team and support

6.1 Contributions of the Team Members

For developing the sensor, all team members have been divided over various subteams (Biochemical Recognition 'BR' and Detection Group 'DG') that have worked together intensively.

Sanne ten Damme	Team Captain and member of BR, mainly responsible for communication with the Sensus organisation and planning
Anouk Baeten	Vice-Captain and member of DG, mainly responsible for Sensus submissions
Melissa Pherai	Treasurer and member of DG, mainly responsible for purchasing equipment and keeping track of the budget
Cathelijne Radstaake	Public relations officer and member of DG, mainly responsible for design of detection device and software development
Giulia Pötgens	Social media administrator and member of DG, mainly responsible for maintaining LinkedIn and Instagram pages of T.E.S.T.
Funmilayo Olugbega	Website administrator and member of DG, mainly responsible for design of detection device and software development
Maurits Overmans	Public relations officer and member of BR, mainly responsible for delivering entrepreneurship documents, maintaining and acquiring sponsorships
Christopher Reenis	Secretary and member of BR, mainly responsible for keeping minutes and designing the T.E.S.T. 2021 homepage
Justyna Piotrowska	Sensus medals officer and member of BR, mainly responsible for biochemical assay design and making Sensus medals assignments
Paul Thur	Prototype developer and member of DG, mainly responsible for software development and design of the *Donut* prototype

6.2 People who have given support

Throughout the year, T.E.S.T. has received support from many people. The people that have contributed the most are listed below.

Leo van Ijzendoorn	Main supervisor of the entire team
Rafiq Lubken	Supervisor of the entire team
Claudia Schot	Supervisor of the entire team

Arthur de Jong	Offering lab-guidance and assistance
Stijn Haenen	Providing crucial assistance in the set-up development
Emiel Visser	Providing useful advice on the assay and in the lab
Khulan Sergelen	Providing crucial advice on the assay (protocol) design
Yu-Ting Lin	Providing essential information on sugar preserving and in-kind contribution
Willem Rover	Providing advice and materials for the detection group
Edwin van den Einden	Providing useful insight on the prototype development
Max Bergkamp	Giving crucial advice and assistance towards the software development

6.3 Sponsors

T.E.S.T. 2021 is very grateful to the sponsors of this year whose support has made this project possible.

TU/e	Financial aid and support
TU/e Innovation Space	In-kind contribution (advice and office space)
Antibodies online	In-kind contribution (antibodies)
Flir	In-kind contribution (camera)
ThermoFisher scientific	In-kind contribution (advice)

7. Final remarks

It has been a pleasure working together with this team on this project. We learned even more than we could imagine at the beginning, including soft and hard skills.

The team thinks that there is a lot of potential in this concept with a lot of possible future research. In the future, some of us might continue this or similar work during other projects or internships. The assay could be improved by doing more experiments with saliva, further optimizing the number of used particles and the amount of functionalization. We are certain that experiments using different biomarkers could also result in desirable outcomes. The biosensors could also be made more automatic and the cartridge could be optimized. In short, there are a lot of possibilities for team members to maybe work on in the future.

We would like to end this document with emphasizing our appreciation once more to everyone who has helped us this year. Especially towards Leo and Rafiq, thank you for everything this year. We could not have done it without you, especially during the summer and finalizing the TRD.

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Appendix

A0 Calculations and simulations of the assay

A0.1 Calculations of number of antibodies per particle

Density polystereen: 1.05 g / cm^3
Volume particle: 4/3 * pi * r^3. = 4/3 * pi * (0.5x1^{-4} [cm])^3 = 5.236 x 10^{-13} cm^3
Mass particle: density x volume= 5.236 x 10^{-13} cm^3 *1.05 g / cm^3= 5.489 x 10^{-10} mg
Mass of antibody per particle (mg): Mass particle x (20x10^{-3} [mg]) = 1.10 x 10^{-14} mg
Amount of antibody = g antibodies / molecular weight of 1 antibody
150.000 gram / mol (average molecular weight of 1 antibody)
Mol antibody per particle = (1.10 x 10^{-17}g / (150.000 gram / mol) = 7.33 x 10^{-20} mol
(7.33 x 10^{-20}) * (6.02214199 x 10^{23}) = **$4.4x10^4$ antibody per particle**
Number of antibodies per particle = antibodies per particle (g) / molecular weight antibody (gram/mol) * number of Avogadro

Assumptions:
- A particle has the same mass as polystyrene
- A particle is perfectly round. (Not the case but for the calculations that is fine)
- A particle can bind 20 microgram of antibody per 1 miligram particle (specifications are online)

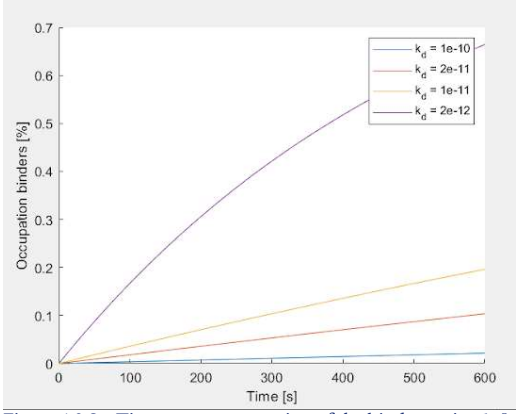
A0.2 Incubation concentrations calculation

Per particle there are = **4.4x10^4 antibodies**

Concentration stock Ab: 10 mg/mL
Dilute 10x to 1 mg/mL

Amount of particles = total amount mg / mg per particle
1 / 5.489 x 10^-10 = 1.821825468*10^9 particles
Amount of antibodies (mg) on particles in total = amount of particles * mg antibodies per particle
1.821825468*10^9 * 1.10*10^-14= 0.00002004 mg
Amount of antibodies used to incubate should be 10x as much so:
0.00002004 mg * 10 = 0.000200401 = 2.004008015*10^-4 mg = **0.2 µg/mL antibody concentration when using 1:1**

A0.3 Figures of the time versus the occupation



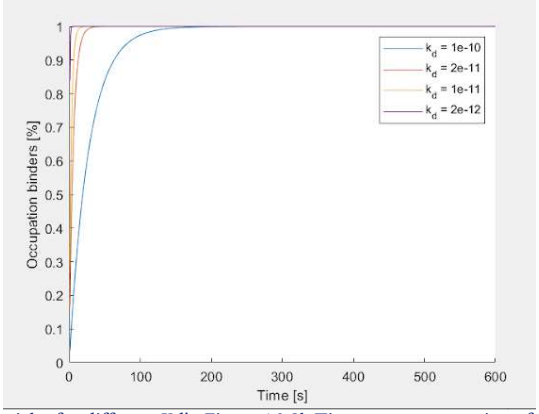


Figure A0.3a. Time versus occupation of the binders using 1e5 particles for different Kd's Figure A0.3b Time versus occupation of the binders using 1e5 particles for different Kd's

A0.4 Figure of coefficient of variation versus target concentration

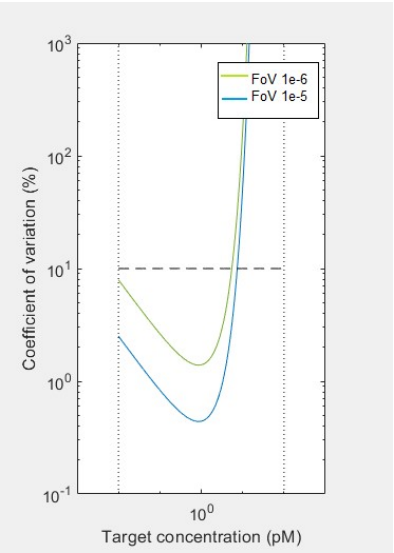


Figure A0.4 Coefficient of variation versus target concentrations for FOV's of 1e-6 m^2 and 1e-5 m^2. In the figure it can be seen that the bigger FOV results in a bigger precision

A0.5 Sugar preservation of functionalized surfaces

Functionalized surfaces could be stored for a few days but needed to be kept in humid environments at 2-8 degrees Celsius. From a production standpoint this expiration time is too fast to ensure consistent and reliable results for every user of our biosensor. Sugar drying the surface (thereby covering the antibodies with a dried sugar solution) was explored to lengthen this expiration rate to 2 to 3 weeks. A sandwich immunoassay was performed on a surface that was sugar dried and kept for 3 days at room temperature. The sugar mix contained 25% sucrose and 10 mM Trehalose dissolved in milliQ water. Drying took place under (full) vacuum for 3 days. The resulting counts of particles of the sugar preserved surface was compared to a surface that was freshly functionalized with the same concentration cAB. The average number of counted particles was very similar. This means that sugar preservation had no negative impact on binding capabilities: the cABs on the surface and the sandwich immunoassay still worked comparably well.

A1 Customer Journey

A1.1 Customer Journey Map High-Risk population municipality services

Jan is a 72-year-old man with obesity (BMI >30) with Asthma as co-morbidity. In the customer journey map, Figure A1.1, the process from awareness through treatment is explored. Additional monitoring done by Jan's doctor for the rest of the influenza season is important to show the benefit of having T.E.S.T.'s biosensor as a rapid, affordable, and easy-to-use POCT diagnostic tool.

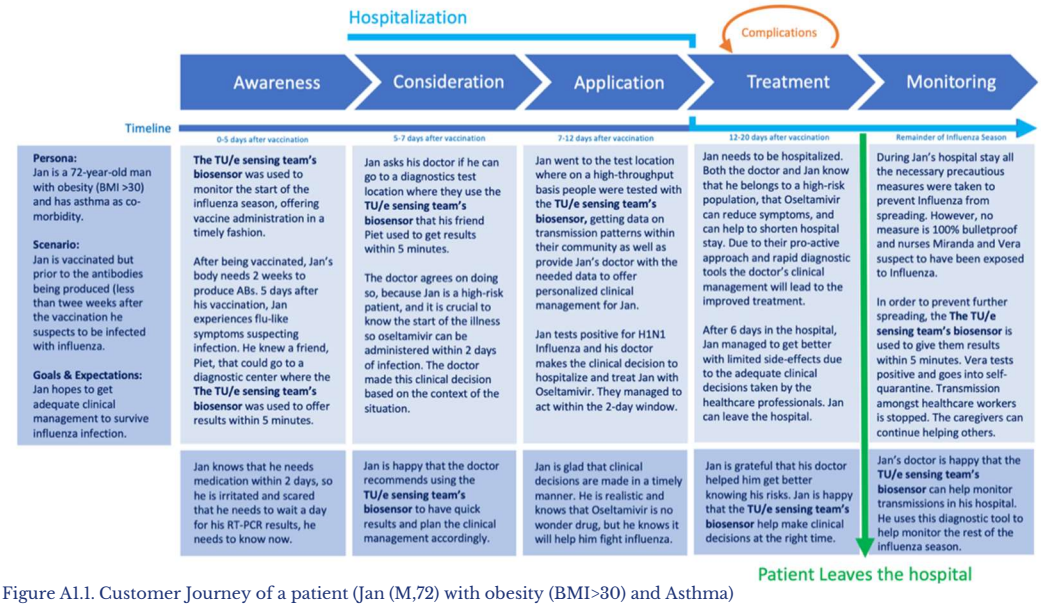


Figure A1.1. Customer Journey of a patient (Jan (M,72) with obesity (BMI>30) and Asthma)

A1.2 Customer Journey events in Influenza season

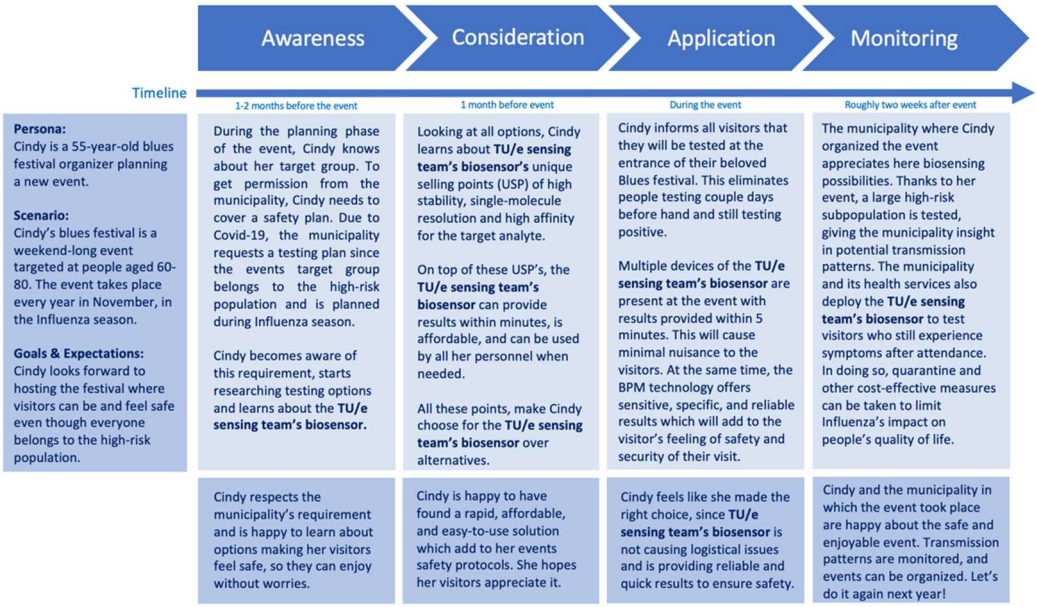


Figure A1.2. Customer Journey of Cindy looking to organize a festival for people aged 60-80 in the Influenza season.

A2 Cost-Utility 2009 H1N1 pandemic

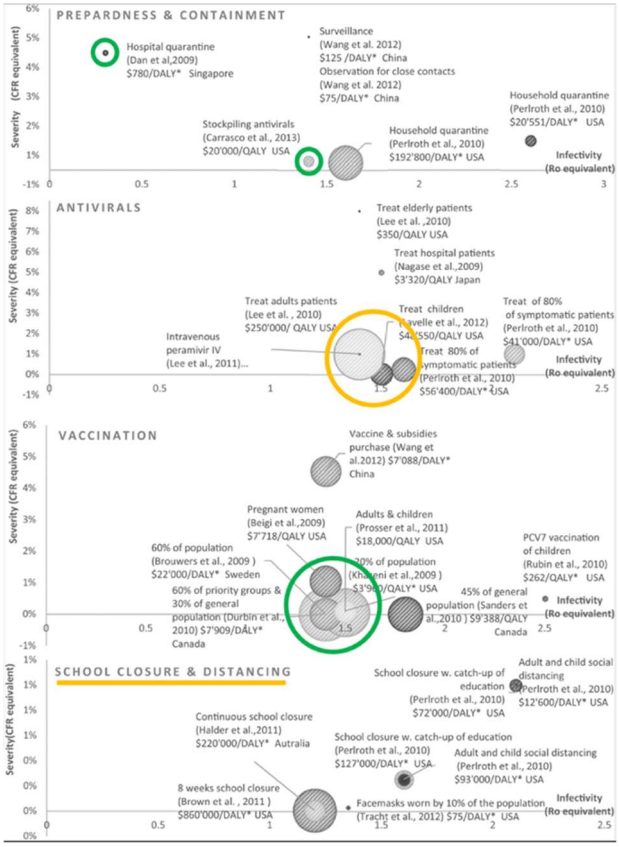


Figure A2.1 Evaluation of the cost-utility of the 2009 H1N1 responses with respect to the severity levels. The cost-effectiveness on the x-axis is expressed in reproduction number (R_0) equivalent. On the y-axis, the severity is expressed in CFR equivalent, this stands for the case-fatality ratio. The overall graphic represents the incremental cost-effectiveness ratios (ICERs) and converted cost-utility measures (CCUM), see Appendix A1.2. The circles are used to indicate individual measures. Additionally, the circle size indicates the cost-utility of a measure in dollars per DALY. Bigger circles correspond to more costly measures per DALY. (Pasquini-Descomps et al., 2017)

A3. Value- and customer profile map

The essence of the value proposition behind the offered core products and services can be seen as an on-going challenge to improve this team's solutions to optimize the product-market fit. Something which is quite a challenge to find and maintain in itself. In order to do so, value- and customer profile maps are used illustrating the pains and gains, the prior without T.E.S.T.'s sensor and the latter with this team's biosensor respectively

Table A3. Value- and Customer profile map. The priority of the individual points is based on the order of the information.

Value Map	Customer Profile Map
Products & Services	Customer jobs
● The core product of the TU/e Sensing Team (T.E.S.T. 2021) is a Rapid Influenza A diagnostic biosensor based on Biomarker monitoring by particle mobility sensing (particle imaging). (Including maintenance) ● Measurement of HA1 protein within 5 minutes in a high throughput manner. ● The cartridges used to measure the samples are one-time use, so long-term relationships build through high-end service. ● On-going research and development to improve the technique and suit the needs of customers to increase the product-market fit.	● Minimize impact of the disease by providing useful information to public health authorities ● Identify and monitor groups of high risk of severe disease and mortality. ● Provide best possible treatment for high-risk patients by having adequate decision-making needed to evaluate treatment options in the two-day optimal medication (e.g., oseltamivir) efficacy window. ● Optimize Influenza vaccine timing by appropriate estimations of the start of the Influenza season.
Gain Creators	Gains Customers
• Diagnose based on inactivated viral H1N1 influenza particle in TBA artifical saliva, biomarker Hemagglutinin (HA1) in a unique manner. • Rapid, accurate, and easy measurements. • Testing concentration of 107-1011 virus particles/mL. This converts to a concentration of 0.01-100 picomol/L, or 1 - 10,000 pg/ml of HA1. • Detect influenza before symptoms appear • Fixed time interval of 5 minutes • Tabletop design: The biosensor system may be no larger than 80cm x 80cm x 50cm. • Cost-effective design • Future personalized medicine options may be possible.	• No need for expensive labs, highly trained personnel, and specialized equipment when using the T.E.S.T. biosensor. • Detect unusual and unexpected events such as outbreaks of influenza outside the typical season, severe influenza among healthcare workers, or clusters of vaccine failures that may herald novel influenza virus. • Better planning of appropriate control and intervention measures. • Better allocation of health resources and make case management recommendations. • Signal the start and end of the influenza season.
Pain Relievers	Pain
● Particle imaging-based biomarker that is affordable, easy-to-use and capable of Influenza A detection within 5 minutes. This is an improvement over competitors. ● Single-molecule resolution in evaluating the biochemical interactions. This is done by the detection device that uses optical imaging in order to simultaneously record hundreds of particles. This offers the needed specificity and precision. ● This team's supporting, maintaining, and improving the unique biosensor concept through on-going research and testing. ● The product is engineered in order to monitor Influenza A to minimize its effect on people's quality of life.	• Misdiagnosing of Influenza A (H1N1) may lead to an underestimation of serious conditions. • On the other hand, misdiagnosing of Influenza A can lead to unnecessary treatment with oseltamivir, resulting in unnecessary costs, patient exposure to side effects and over implementation of infection control procedures in hospitals. • Lack of an established surveillance for severe diseases in most countries Limiting mapping the severity • Lack of a pre-existing international mechanism for sharing epidemiological data challenges to understand global patterns of transmission and disease. • The existing studies suggest that hospital quarantine, vaccination, and usage of the antiviral stockpile are highly cost-effective, even for mild pandemics. Non-optimal monitoring will require unnecessary expenditures to bring the virus back to controllable levels.

A4. SWOT analysis

Knowing the external factors affecting the translation of the T.E.S.T.'s ideas into products is only one half of the equation. The other half, assessing the team's current position, is important in determine the strategy needed to enter the market in an effective manner. In order to do so, a SWOT analysis is used. SWOT stands for strengths, weaknesses, opportunities, and threats and is used to assess this aspect of this team. In doing so, the most can be made out of this team's advantages as well as a reduction of the chance of failure. In understanding this team's lacking skills or resources, a strategy can be created to find a competitive advantage, be pro-active in identifying hazards, and work on strengthening the core team. As a result, successful market entry will have a higher chance of occurring. The individual points made in all four categories are numbered to assign priority of relevancy.

A4.1 Strengths

1. T.E.S.T. is proudly representing their university, with great ambition, competitive spirit and all the time needed to make this sensor a success. The remainder of the time with T.E.S.T. will be spent researching and implementing an innovative biosensor. This team and the dedicated time for this project is the biggest asset.
2. All team members are educated in biomedical engineering (BSc and MSc students), however all members have followed different courses and experienced different backgrounds. This adds to the diversity and provides for greater value in the interdisciplinary team structure.
3. The Helia and MBx group developed particle imaging, combined with the patents, giving full expertise within this (sub)field, knowing the right contacts and providing for a very strong pioneering position in the biosensor field
4. The detection method for particle imaging does not require extensive electrical engineering skills and is way more cost-effective than electrical impedance spectroscopy, offering greater value provided by us as a team and a way value friendlier solution.
5. Helia as a company combined with the entrepreneurial ambitions and experience of this team makes for the design of a product that has good translational potential, meaning that it can reach a large customer pool improving more people's quality of life.

A4.2 Weaknesses

1. All members are biomedical students, not having people with different disciplines like electrical engineering or computer science makes us vulnerable in those sections of designing the biosensor.
2. After being done with T.E.S.T., people have had enough and do not like to continue. Breaking the team up and decreasing the time and skills that can be brought to the table for a collaboration.
3. This team is "too lightweight" to optimize the revolutionary particle imaging into rapid, affordable, easy-to-use biosensors within the specified timeframe of 5 minutes, offering little towards the goals with Helia to innovate into measure sensors for virus pandemics.
4. Team T.E.S.T. cannot possibly do high-scale production, assumed that Helia also needs a third party to do the production when the technique of particle imaging is brought to the market. Outsourcing production can be costly, and when the wrong partner is used can lead to the copying of the technique just different enough to bypass the patents.
5. The timing of market entry is in a " low period" where there is no possible pandemic looming around and the bigger parties don't see the need to buy the products.

A4.3 Opportunities

1. The need for closed-loop control of healthcare and industry is increasingly growing with the current pandemic, adding to the need for new innovative approaches to bring point-of-care sensors to the market. The desire for personalized POCT keeps increasing for a long-term period.
2. Engineering a particle imaging sensor offers a rapid, affordable and easy-to-use method reducing the need for large staff, expensive equipment or laboratories lowering the high expenses on healthcare.
3. Detection time of < 5 minutes is achievable with particle imaging offering great chances to optimise the technique for point-of-care testing with Influenza A.
4. Both Helia and the MBx group are heavily invested in continuous monitoring. The covid-19 pandemic is showing the need for sensors to give rapid results whether a person is infected or not in order to make decisions and improve the people's quality of life. This provides for huge potential to innovate.
5. The technique is patented by Helia, providing for great possibilities to add on this technique together with the experts who pioneered this revolutionary sensing method.

A4.4 Threats

1. Particle imaging as this team's intended method combined with viral measurements has no mentioning in any reports, nor is it tested with the application in Influenza A, therefore making it able to innovate but it does also possess the threat of failing.
2. We are one of the two main groups at the TU/e working towards biosensors, therefore are resources such as funding, exposure and experts spread over these two groups. The group of Prof. Dr. Merkx and his LUMABS sensor is already working in the covid application for rapid testing, followed by many competitors who see this "blue ocean" as an opportunity to enter and take over the market of point-of-care rapid testing for viruses like influenza A. Big companies with large capital can be very dominant in the market area, making market entry difficult.
3. Doctors and first-line care providers would prefer current detection methods, like RT-PCR, to obtain more than just a diagnosis, making the biosensor unnecessary and losing potential customers.
4. Just like we see with Covid-19, education on the technology can be too vague or too difficult causing the potential users to doubt the intentions and effectiveness of the technique, therefore stigmatizing POCTs and fewer companies will invest.
5. The distribution channels of point-of-care biosensors, like the particle imaging, can be okay in the Netherlands, but internationally there are big irregularities in operating procedures of POCT's in Europe alone.

A5 Financial calculations

A5.1 Cost Calculation Cartridge

Table A5.1 Overview of the total costs of cartridge

Object	Price / unit	Needed per cartridge	Price / cartridge
Dynabeads® MyOne™ Streptavidin C1 Dynabeads™ MyOne™ Streptavidin C1 (thermofisher.com)	554 (per 2 mL)	0.01 mg/mL	0. 0.00277
Microscope glass slides Rogo Sampaic™ Glass Microscope Slides: Glass Microscope Slides and Coverslips Beakers, Bottles, Cylinders and Glassware | Fisher Scientific	2.50 (per 50 pieces)	1 per cartridge	0.05
Flow cell stickers Grace bio-labs Custom SecureSeal	10 dollar per 6 cells 8.52 euro per 12-08-21	1 per cartridge	1.42*
Anti-HA1 2009 Capture antibody Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA ELISA Pair Set | ABIN2010157 (antibodies-online.com)	470 euro (per elisa kit) 235 euro (per 1 mg/mL)	mL 62.5 ng/mL	0.188
Anti-HA1 2009 Detection antibody Influenza A H1N1 (Swine Flu 2009) Hemagglutinin / HA ELISA Pair Set | ABIN2010157 (antibodies-online.com)	235 euro (per 0.2 mg/mL)	0.8 ug/mL	0.073
ChromaLINK® One-Shot™ Antibody Biotinylation Kit B-9007-009 ChromaLink® One-Shot Antibody Biotinylation Kit (vectorlabs.com)	377.30 euro Per 10 mL biotin-Ab	25 uL	0.94325
PEG-biotin Biotin PEG Derivatives, Biotinylation Reagents-Biochempeg	153.31 euro (per 1g) AS OF 11-08-21	500 uM	0.0765
BSA Bovine Serum Albumin lyophilized powder, crystallized, ≥98.0% (GE) | 9048-46-8 (sigmaaldrich.com)	85.70 (per 1g)	100 mg per ml 2.5 mg per 25 ul	0.21425
TOTAL			2.8885

*This cost might be reduced by means of injection molding.

A5.2 Cost Calculation Prototype

Table 5.2 Overview of the total costs of prototype

3d print material	€100
3d print production costs	€70
Camera	€ 480
Light tube	€ 142
Objective	€ 80
Translation stage	€ 216
LED light	€ 30
LED mount	€ 60
Microscope clamps	€ 56
Total	€1234

A5.3 Production costs estimation

Some of the prototype costs might be reduced by means of upscaling and injection molding, we estimate these costs to be 10% of the total costs of the prototype. The labor costs are estimated 10% of the price of the raw production materials (Europe). For marketing and distribution another 10% costs per device is estimated (Europe).

Table 5.3 Overview of production costs of prototype

Device costs	€123
Labor costs	€12
Marketing and distribution	€12
Total costs per device	€147

A5.4 Sales estimation in the Netherlands

Table 5.4 Overview of sale estimation

Sold		Costs	
Devices sold to municipal health services	€50,000 (100x€500)	Total device production costs	€99,960 (680x€147)
Cartridges sold	€290,000 (38,667x€7.50)	Total cartridge production costs	€11,600 (38,667x€0.30)
Profit	€228,440		

A6 Social Impact

At T.E.S.T. we are excited to be working on the biosensing based on particle mobility (particle imaging) principle in order to bring rapid, affordable, and easy-to-use biosensing solutions to detect Influenza A. In doing so, we aim to contribute towards better planning of appropriate control and intervention measures, better allocation of health resources, and lastly improve case management recommendations. This leads to improved identification, monitoring, and help for groups of high risk of severe disease and mortality. Additionally, the improvements can lead to increased patient access to treatment and advice worldwide. As a result, on-going enhancement of research and mobile health can contribute towards democratizing healthcare worldwide.

Knowing the proven track record of the MBx group with the particle imaging technology, this team is confident that the unique biosensor offers substantial potential to contribute towards improved pandemic healthcare. Combined with the enormous demands of rapid, affordable, and easy-to-use point-of-care biosensing solutions for Influenza A, a high level of societal impact can be achieved.

All in all, the products/services offered by T.E.S.T. works towards solutions for the sustainable developmental goal 3: good health and mental well-being. In doing so, this team works towards two of the subgoals defined by the United Nations in 2015.(Duurzaam Regeerakkoord, 2020) These being:

- Having the capacity to strengthen all countries, and especially those of countries in development, with regard to early warning systems, risk reduction and management of national and global health risks. This is where biosensing is needed as explained in the section "nature of the problem". (Duurzaam Regeerakkoord, 2020)
- The aim is to end epidemics in 2030 like Aids, tuberculosis, and malaria amongst many other tropical diseases. In doing so, monitoring the spread of these diseases is of fundamental value. Rapid, affordable, and easy-to-use biosensors needed to do so. (Duurzaam Regeerakkoord, 2020)