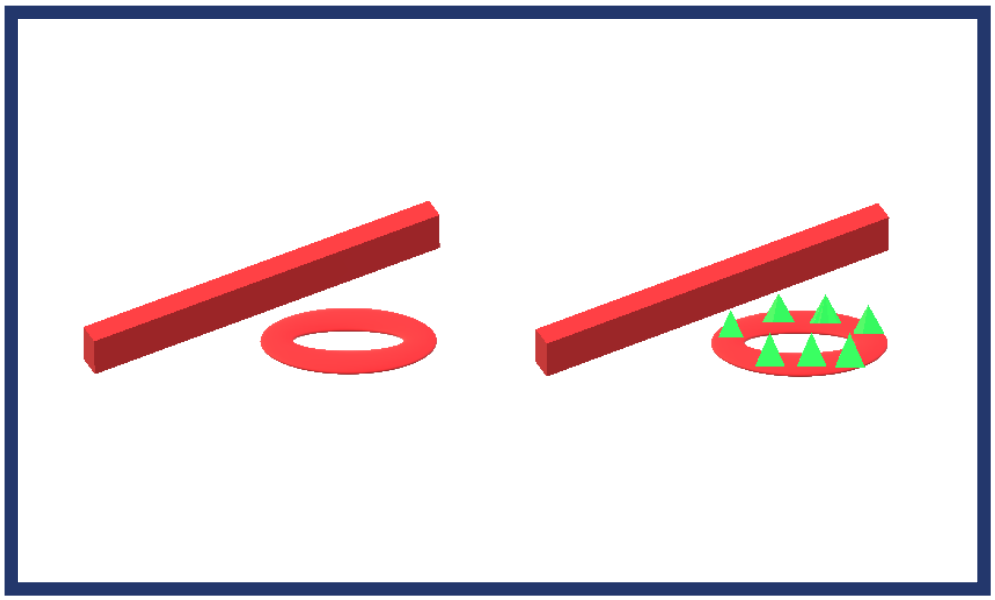
Team Results Document

UT+



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17-08-2021

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SensUs

1. Abstract

The UT+ rapid test for Influenza A detection is a photonic biosensor, which utilizes microring resonators to detect the analyte. This type of laser sensor is highly sensitive to small changes in concentration of the target molecule. With automatic chip-aligning and data self-collected-and-processed set up, this sensor has a high potential to access the market.
With this product, we aim to firstly target the veterinary sector, in which influenza is a big problem in zoonotic infection, especially from swine. As clinical trials and certifications are not required in veterinary applications, a quick entry in the market with an accelerated revenue would be possible. With this income, we can continue with the clinical tests to release a safe product in the human healthcare market. In parallel, we also aim to tackle the research-only market, which might be a smaller but easier-to-access market, with less requirement in certification and trials, which would also enable us to obtain application data (publications), in coordination with future IP. More importantly, our sensor can be easily adapted to detect other viruses demanded from various markets; and with a multiplexing chip, the cost of every test and the waste can be minimized.

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

The UT+ biosensor to detect Influenza A is a photonic biosensor, using microring resonators integrated on a chip. This type of sensor is not very commonly used for biosensing but has a lot of potential due to its high sensitivity to small concentrations [1][2][3][4][5].

2.1 Molecular recognition and assay reagents

On the surface of the chip a material-selective antifouling layer is deposited. The chip is made from two different materials. The waveguides and microring resonators (MRRs) are made from Al_2O_3 on a SiO_2 substrate andrest of the surface of the chip is SiO_2. To create the antifouling layer, the chip is functionalized with 12-amino-dodecyl-phosphonate followed by mPEG-propyl trimethoxysilane. The phosphonate is selective towards the Al_2O_3, therefore it is expected to mostly bind on the waveguide and rings. After this NHS-PEG_4-Biotin is added, which binds only to the amines on top of the phosphonates due to functional group selectivity. Next, the mPEG-silane is functionalized on the rest of the chip. Thereafter streptavidin is bound to the biotin. Finally, the biotinylated antibody is added, which binds to the streptavidin. This way only the waveguides and ring resonators will be functionalized with the antibody. To make the detection more sensitive, down to 1 pg/ml, an amplification step was included. Polystyrene beads of about 400 nm coated with streptavidin and biotinylated antibody are used as amplifier.

2.2 Physical transduction

The biosensor presented by the UT+ team is an optical sensor [6]. A chip with different integrated Micro Ring Resonators (MRR) is used. In the MRR, the light from a laser source is coupled into the bus waveguide. The materials that are used for the waveguide and the cladding layer have different refractive indices so the light becomes confined in the waveguide [7][8][9]. This phenomenon happens when the angle of refraction is higher than the critical angle. Hence, the light will not be able to get out of the waveguiding material. The ring acts as a filter and it resonates at specific wavelengths. This is due to the wave's interference: every point where there is constructive interference (a resonant wavelength) happens once every round trip, and its path length is exactly a multiple of the wavelength. When light of the resonant wavelength is coupled in, it enters the ring and builds up in intensity while doing multiple loops. While the light is in the ring a dip in the power intensity in the output can be detected (one dip for each loop the light does).

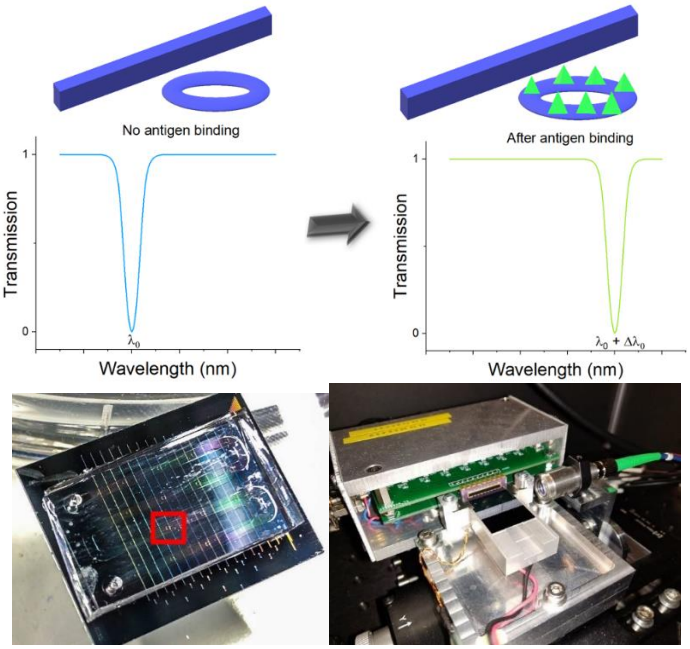

Fig.1 a. Biosensing principle, b. Rings on the chip, with 1 PDMS channel, c. Experimental setup.

These resonant wavelengths are dependent on the refractive index of the surroundings of the MRR. Photonic integrated biosensors [7] take advantage of the evanescent field surrounding the waveguide, which influences the sensing performance. The principle for considering the evanescent field is that the refractive index (n) in a specific point on the waveguide is correlated to the resonant wavelength position. This explains why we observe a shift on the resonant wavelength when the refractive index on the waveguide changes. The refractive index is dependent on different factors such as temperature or the presence of a target molecule at the surface. Binding molecules to the MRR make the peak (resonant wavelength) shift proportionally to the molecule concentration (Fig. 1a). Thus we can determine how much of the target molecule (antigen) we have with very high accuracy. To improve the limit of detection an amplification step is used. This step includes the use of polystyrene beads, which are nanoparticles with a size of 400 nm. These beads are much larger than the antigens and therefore amplify the refractive index change measured by the system. In other words, as the beads are much bigger than the antigens, when they bind to the surface of the MRRs they will generate a larger change in the refractive index compared to the binding of the antigen alone. A larger change in refractive index means that the shift in the resonant wavelengths will also be much larger, so more easily measured by the system.

2.3 Cartridge technology

For this biosensor, SiO2 chips with Al2O3 waveguides are used. The chips used have multiple rings integrated as shown in Fig. 1b. Having multiple rings allows for self-referencing and multiplexing. For the competition, multiplexing will not be used (not allowed) but having multiple rings on the same chip permits using one chip for more than one sample. This lowers the costs and the waste. The samples need to be flown over the MRR to detect the antigen present in them. During initial prototyping stages, microchannels are patterned on a mold on which Polydimethylsiloxane (PDMS) is cast. Then, a piece of PDMS with the patterned channels is bonded to the chip surface. The PDMS is first activated and bonded to the chip by placing it on top and applying a slight pressure. Before the bonding of the PDMS, inlet and outlet holes are punched at the inlet and outlet of the patterned microchannels. For the samples and solutions to flow through the channels, a tubing system is used. The tubing system is connected to the different samples and solutions, which are stored in 2 mL vials (samples) and 10 mL vials (buffer solution). There is one tube that is connected to the samples or buffer solution and the inlet, and then another tube connected to the outlet of the PDMS and a waste syringe. There is a negative pressure flow controlling system that was set to a flow rate around 3 ml/h. This flow control system consists of a syringe pump connected to the system. By sucking with the syringe, a negative pressure reservoir is created.

2.4 Reader instrument and user interaction

The set-up consists of different parts. The chip is placed on the platform, which performs auto-alignment (Fig. 1c). This platform has the laser source attached to one side, corresponding to the inlet side of the waveguides on the chip. On the adjacent side of the chip platform there are photodiodes placed. These photodiodes correspond to the outlet of the waveguides on the chip. The photodiodes will detect the light coming out of the waveguides. In the setup there is also a temperature controller to ensure temperature stability. The whole system is connected to a data acquisition system, which is connected to the laptop and controlled by LabView to extract the data for analysis.

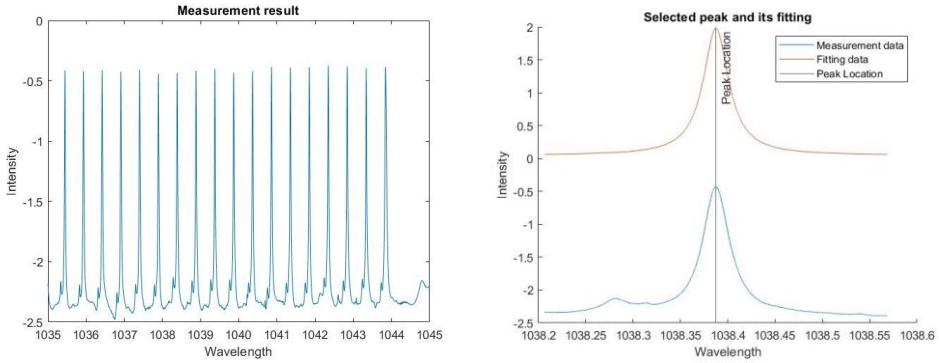

Fig. 2 a. Data of one measurement, b. Fitting of a selected peak from this data, c. User interface.

For the data analysis, a custom-written MATLAB script is used. More details about the script can be found in Appendix A (main script for data analysis) and Appendix B (user interface).

3 Technological feasibility (max. 2 A4)'

3.1 Characterization Experiments

Since the chip is very sensitive to a refractive index change in the liquid, it is also very sensitive to changes in refractive index due to temperature. An experiment to test the temperature stability was carried out. For this experiment, the temperature was increased by 1 ºC steps while the measurement was running, in a range from 22 to 32 ºC. In Fig. 3, the results from this experiment are shown.

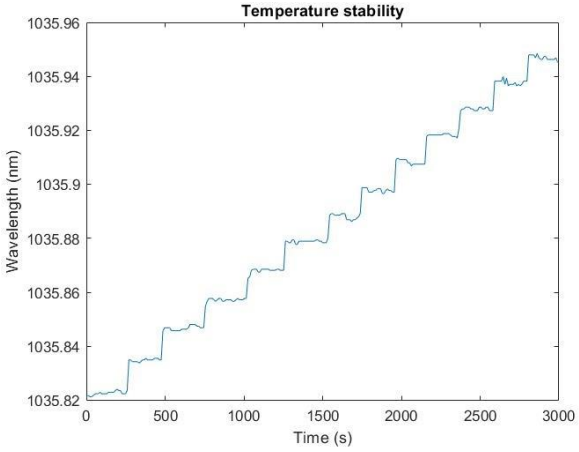

Fig. 3 Plot of temperature stability measurement, with the wavelength in the y axis and the time in the x axis.

As it can be seen in Fig. 3, the resonant wavelength shifts in clear steps, showing a stair-like plot. Every single step has a flat profile, which ensures the temperature is stable and accurately controlled by the system. According to our measurements, the thermal sensitivity is 10 pm/℃, which is small enough since we control the temperature about 20 fm/℃. Overfitting of the Lorentz curve due to the noise from the data has already been filtered out from the plot.

Fluorescence experiments were performed. A qualitative analysis of specific binding of streptavidin onto the MRR and waveguide using fluorescence microscopy was done. In Fig. 4 a fluorescence image of a MRR and its bus waveguide can be seen.

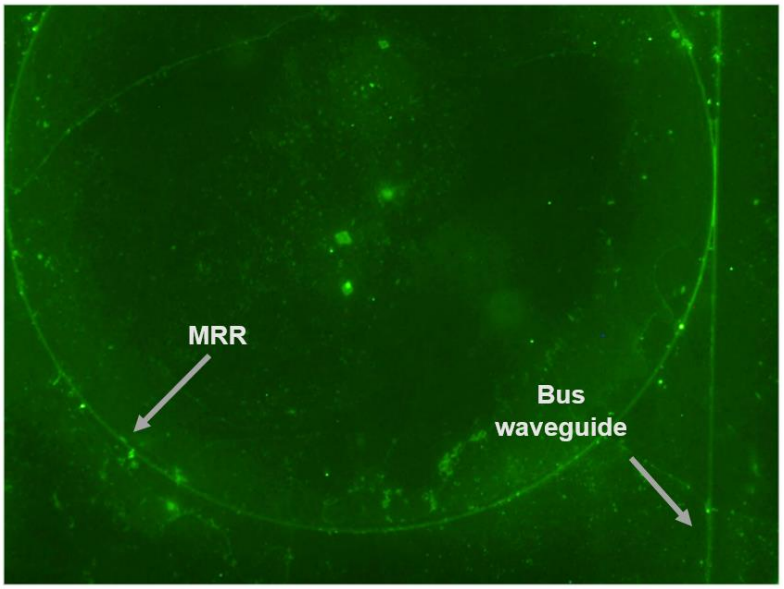

Fig.4 Fluorescence image of surface modified biosensor chip

From the image in Fig. 4 it can be seen that the fluorescence is concentrated on the MRR and the waveguide, which are the sections that are supposed to be functionalized with phosphonates selectively. With this test we can verify that the phosphonates are indeed selective to the Al_2O_3.

3.2 Influenza Experiments

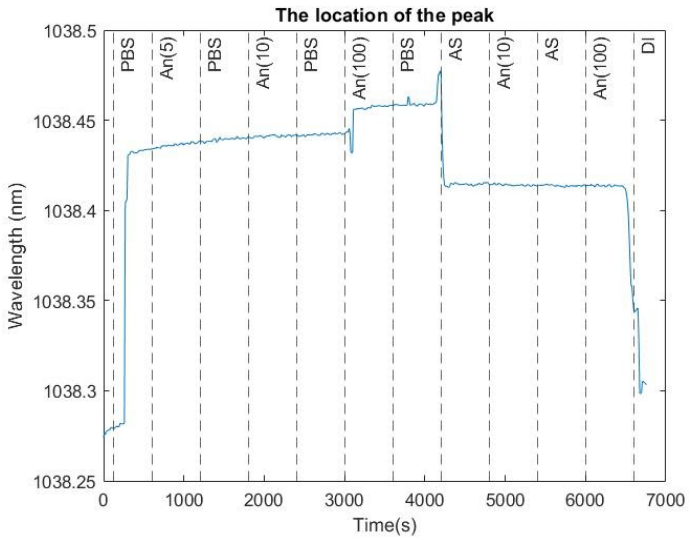

Fig.5 Plot of a biosensor response with various antigen concentrations having measurement number in x-axis and wavelength in y-axis

In Fig. 5, the measurement was started with flowing the DI water into the PDMS channel on the chip. After a few minutes the flow was shifted to PBS, which can be evidenced from the graph as a huge jump due to change in the refractive index of the medium. There is a lag in the data due to delay in fluids reaching the chip from the vial. Later, the flow was shifted to PBS with an antigen concentration of 5 ng/mL (An (5)) followed by increasing concentrations of antigen as indicated in the graph. Only a small binding curve at a concentration of 100 ng/mL of antigen can be seen but anything less than this cannot be visually seen due to dominance of thermal noise. To make our biosensor more sensitive for the required range (1 pg/mL - 10 ng/mL) an amplification step will be used. For amplification PS beads of about 400 nm coated with streptavidin and antibody will be flowed after

injecting the sample through the PDMS channel. Due to their much higher mass and similar refractive index, an amplification of up to 106 is expected. It is believed with this amplification step the sensor limit of detection can go down to 1 pg/mL, or even 100 fg/mL, but for this to work properly the right concentration of amplifier needs to be chosen.
From the second part of the graph on, the antifouling property of the surface against artificial saliva (AS) was recognised. After introducing the AS there is a big dip in the graph due to change in refractive index. Since the shift is huge, another resonance peak exactly overlaps the current position, and this is the reason for a dip instead of a jump as observed with PBS.

4 Originality (max. 1 A4)

4.1 Team Captains

To detect Influenza A in a sample of artificial saliva, we designed a biosensor that makes use of optical sensing. Optical sensing is not a commonly used method in a biosensor, which makes our biosensor more original. The use of antibodies for molecular recognition is more standard. In combination with light, however, it is an innovative principle. In our biosensor, we make use of a photonic chip with MRRs. The setup used was created by a research group from the university (the Optical Science group) but adapted by the UT+ team. Additionally, for the antifouling layer, a selective functionalized layer was developed by the team in collaboration with the Molecular Nanofabrication group from the UT. The flow control system that was being used by the Optical Science group was too big and too complex for the setup our team was developing for the competition. Therefore, the fluidics system was adapted by the team in order to make it more functional and optimal for our particular setup. About the software for the data analysis, the Matlab script used was written by our team, to make it specific and practical for our sensing system and the type of data. Also, the box where the setup is placed was also designed by our team.
Besides, the team also did an intensive networking job with different people inside the university and with different companies that helped with expertise. It was also a valuable addition to the project the fact that different stakeholders (from fields ranging from veterinary field to venture capital) could be interviewed who helped with the business case.

4.2 Team supervisor

The UT+ team 2021 has decided to work on optical micro ring resonators in combination with molecular surface functionalization. To do this, the team has established collaborations with two research groups in the MESA+ Institute for Nanotechnology (University of Twente): Optical Sciences and Molecular Nanofabrication. In Optical Sciences, the research group had been working in a similar concept but that was developed for COVID-19 detection. The team has then adapted the platform for Influenza A testing and added the possibility for parallel measurements of up to 4 samples. To make this work, they have set up a collaboration with Molecular Nanofabrication to develop a customized surface functionalization approach specific for Influenza A. The merits of the team have been in scanning existing research within the UT and industrial partners to envision the leanest path to solve the problem. We believe that this made sense as the team consisted only in 4 undergraduate and 4 master students. In addition, they intensively networked with the Twente industrial ecosystem to obtain key advice in technical aspects (e.g. electronic interfacing from 3T, microfluidic dispensing from Micronit, medical device business development from Demcon and flow control from Bronkhorst). It is also worth highlighting that the team accomplished to collect feedback from a variety of stakeholders (clinical,
regulators, MedTech developers, veterinary, etc.) which has helped to shape the vision for the biosensor.

5 Translation potential (max. 3 A4)

5.1 Business model canvas

<table>
<tr>
<td rowspan="2">Key Partners
1. MESA+: provide funding sources, micro-fabrication in nano-labs, R&D support and access to a wealth of expertise.
2. Royal GD: support vast knowledge in animal health and data transmission.
3. GGD: can spatially map infections after receiving digital results.
4. Micronit: support in microfluidic dispensing and cartridge design.
5. Bronkhorst: support in flow sensing and advanced microfluidic pumping.
6. Demcon: advice in business development for medical device technology.
7. 3T: support in interfacing electronics and Instrumentation.</td>
<td>Key Activities
1. R&D: System integration and miniaturisation, route to certification, technical improvements in accuracy, capacity, speed, reduce manufacturing costs and adapt the platform to other diseases.
2. Clinical setting for validation: Ensure safety and effectiveness of medical devices and creates transparency for end users. Use application data from the “research use only” phase.</td>
<td rowspan="2">Value Propositions
1. Fast and painless sample collection for humans and animals (saliva based).
2. Avoid laboratories for testing, discarding costly logistics (sample collection & handling, lab running costs).
3. High throughput screening, ideal for disease outbreak control.
4. Short time to get the result, within 20 minutes.
5. Low cost per test (manufacturing costs in between 10 to 20 €).
6. Adaptability to test other diseases.
7. Embedded electronics to safely transmit the test result to a centralised electronic database (additional revenue stream).</td>
<td rowspan="2">Customer Relationships
In the 1st stage, our customers are swine farmers in the Netherlands and Germany. We will reach them via Royal GD Animal (NL) and the Federal Office for Consumer Protection and Food Safety (GE). When expanding we will look at their equivalents in Europe via EU bodies. In between we will supply academic research groups for “research use only”. We will use UT contacts to identify active groups in NL and EU.
In the 2nd stage, we will work with hospitals, nursing homes and elderly homes. They will be reached by GGD (NL) and German Public Health Insurance Organisations (Krankenkassen). When expanding to other EU countries we will work with their equivalent national organisations.</td>
<td rowspan="2">Customer Segments
Stage 1: Animal Market
1. Farms (NL, DE and EU)
2. Research groups in Influenza (human and animal) for the “Research use only” case
3. Royal GD Animal
Stage 2: Animal Market & Human Market
1. Hospitals & Medical Centers
2. Insurance Companies
3. Nursing Homes & Elderly Home
4. GGD (Human)</td>
</tr>
<tr>
<td>Key Resources
1. Human capital (engineers, scientists, technical medicine specialists, business developers, health professionals)
2. Funding (to develop and assemble the biosensor, clinical validation, certification, adaptation to other applications)
3. Laboratory
4. Raw material (artificial saliva...)</td>
<td>Channels
1. National regulatory bodies (i.e., GGD, Royal GD Animal health in NL)
2. Key opinion leaders (infectious disease medical doctors with authority in the field)
3. Conferences, trade shows, professional events & specialized magazines.
4. Direct stakeholder networks</td>
</tr>
<tr>
<td colspan="3">Cost Structure
1. Primary costs are related to R&D such as labour costs, lab and R&D, consumables, certification.
2. Secondary costs arise from setting up a whole marketing and supply chain.
3. Other costs include IP costs (filing and maintenance) and assembly costs.
4. Sourcing through key partners allows us to save the costs in the above.</td>
<td colspan="3">Revenue Streams
1. Primary revenue streams come from selling the biosensor setup, consisting of instrument readers and disposable cartridges to the customers.
2. Business model will be based on supplying the cartridges. Maintenance contracts and exclusivity will be tied to the instrument readers.
3. Adapt the platform to other infectious respiratory diseases (or licensing to other developers)
4. Databases on disease spread modeling (during outbreaks).</td>
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5.2 Market description

Influenza is considered one of the most serious infectious viruses in the world. In the past century, humans have suffered several Influenza pandemics (e.g. Spanish flu in 1918, Hongkong flu in 1968, Russian flu in 1997 and flu pandemic in 2009) [10]. All influenza types originate in wild waterfowl and may be transferred through animals to humans. Among them, pigs and poultry are particularly at risk. To prevent an influenza pandemic in the future, we are developing a rapid testing biosensor which can detect Influenza A [11]. We are envisioning a path to the market in several stages, which allows for early generation of funds to support the whole development path. In the first stage, market entry, we aim to supply the veterinary sector to test influenza in animals. Pigs readily bite into saliva collectors in their enclosures, allowing for easy non-invasive testing that closely resembles the practice in human healthcare. Tracking the spread of diseases among animals that are in close contact with people is the key to avoid future pandemics [12]. This will allow us to start generating revenue while working on the certifications and clinical trials needed to supply the human market. The veterinary market can be reached much faster than the human market because for animal applications no human clinical trials and certifications are required.

In order to reach a considerable market size we first target the Netherlands and Germany. There is a large market for testing in pigs in those countries: in 2020 there were 12 millions pigs in total in the Netherlands and 24 million in Germany [13]. Currently it is not clear how influenza is transmitted within animals, therefore the test presented could help to map the transmission. This centralized database can result in an additional revenue stream. Plans are to continue expansion through the EU area afterwards, with North America and Asia in sight for further expansions. In parallel to this stage, we intend to supply devices for human influenza detection as “research use only”. This has the advantage that clinical trials and CE-certification are not required. The research use market will help to start generating funds and above all it will help to generate application data from human patients, demonstrating the efficiency of the device and benchmarking it with gold standard methods. Hence, this first stage finishes with an established business in veterinary testing and application data (publications) in human influenza detection, paving the way for stage two.

In the second stage, during the human implementation of the biosensor, we will start in the Dutch and German market. Germany currently has 18.14 million elderly, accounting for 21.7% of the total population [14]. According to the aging of the population, this percentage will increase. The EU regulations will facilitate the market expansion to the rest of Europe. Eventually, when meeting the requirements of FDA regulations we will target the United States [15]. This is the same path of expansion as suggested by Venture Capitalist Cottonwood that seems as a reasonable path to follow according to the neighboring countries, similar working culture, gateway to EU and sufficient market size. In further expansion stages we will also consider China and the rest of the world.

5.3 Stakeholder desirability

In terms of detection, PCR (Polymerase Chain Reaction) is the most common method and the gold standard for detecting Influenza A due to its high sensitivity, specificity and reliability [16]. However, PCR also has disadvantages: it takes a relatively long time to obtain results, usually a day, and these tests are quite expensive (€40-50) because of the many steps to be taken [17]. More precisely, PCR is logistically challenging as samples have to be collected on site to be then carefully transported to a laboratory for analyses (usually refrigerated), also resulting in high transport and labor costs. In infectious diseases, these 24 hours can make a big difference as the patient needs to keep isolated to stop potential spreading. Therefore, detecting influenza A by PCR is not the most effective way to prevent spreading of the virus, leaving room for a new generation of biosensors to fill in.

Gerdien van Schaik, a Professor in Epidemiology from Utrecht University and Head in Epidemiology in Royal GD, pointed out that using PCR to detect influenza in animals is similar to detecting human influenza. However, in testing animals, Royal GD test herds instead of individuals because of costs. In veterinary applications, costs per test are a very important factor. Gerdien mentioned that taking saliva as a sample for detection is a good method because it saves pain and it is easy to collect. There are also other diseases that with multiplexing could also be targeted. For instance, Erhard van der Vries, a senior virologist at Royal GD, indicated that along with influenza, Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) are very important in pigs and the for exporting pork meat a PRRSV certificate is needed (Appendix D)[18]. We will consider in the future multiplexing this disease to offer a combined influenza/PRRSV test to supply the market.

When compared to PCR, we can provide a result within 20 minutes, on site, without any complicated sample preparation, with embedded electronics to safely transmit the test result to centralized databases, and with a cost per test estimated to €10-20. The improvement of current detection methods can strengthen farms and hospitals by detecting onsite instead of transporting samples to laboratories, after which test results are transmitted digitally to a central body (e.g. Royal GD in case of animals, GGD in case of humans) that can then spatially map infections. Digitalizing test information facilitates communication to the testers as well: test results and appropriate advice can be shared with the tested through telecommunication.

5.4 Business feasibility

We have collected feedback from key stakeholders including potential customers, regulators, venture capitalists, healthcare innovators and potential partnering companies. The feedback collected (Appendix D) suggests that the business plan is viable.

An important part of the key activities is the clinical validation, which is a key step from animal to human applications. Clinical trials are to prove that the methods are safe and effective in humans and, very importantly, that the new method is superior to existing methods. This clinical evidence is required to comply with the European Medical Device Regulation (MDR), a regulation to ensure safety and effectiveness of medical devices and creates transparency for end users. A great advantage of the EU-wide regulation is that it facilitates entering a large market [15]. Additionally, marketing allows us to reach customers and increase awareness, to thereafter collect feedback from customers. We will expand our network through websites, conferences, trade shows, professional events and specialized magazines. The recognition or promotion of the product by key opinion leaders, such as medical doctors in the field, can greatly expand the audience of the product.

Currently, we are sponsored and mentored by the MESA+ Institute of Nanotechnology, University of Twente.

MESA+ provides us with funding sources, micro-fabrication in nano-labs, R&D support and access to a wealth of expertise and know-how. In addition, we also work closely together with companies with the ecosystem based in Twente. These include 3T, Micronit, Brokhorst, Royal GD Animal Health and Demcon. Among them, 3T provides us with support for interfacing electronics and instrumentation, Micronit supports in microfluidic dispensing and cartridge design, Bronkhorst provides support for flow sensing and advanced microfluidic pumping, Royal GD Animal Health provides vast knowledge related to animal health and Influenza A virus testing, and Demcon provides advice on the development of medical device technology business. We have also had conversations with technology venture capitalists Cottonwood to explore funding needs.

5.5 Financial viability

Keshen Mathura, a seasoned healthcare startup developer and now lecturer in Health Technology Implementation at the TechMed Centre, University of Twente, stated that indicative (based on his experience) commercial costs (if outsourced) will be approximately 100 – 200 € and commercial costs (if developed inhouse) will be approximately €60 - 80k. Similarly it is likely to cost €40 – 50k to get CE certification within approximately 9 - 12 months (minimum 6 months). We took this into account when suggesting a market entry in the veterinary sector.

The timescales to reach the market include 6 months for preclinical evaluation, 6 months for clinical testing and 6 months to a year for CE certification (estimated 2 years to reach the market) with a minimum of 100k€ investment, which could easily be increased to 250k€. It is possible to avoid CE marking if it is an investigational device (research use only). This reduces the market size, but allows start generating income early and, more importantly, generating application data that demonstrates the technical claims of the device, which can be managed in coordination with IP management. Hence, an attractive strategy would be to use the device in research parallel to the implementation of the device in the animal market. If we set the price of the biosensor for each test at about 30 euros, we can achieve a lower price than the PCR test, and at the same time get a considerable profit for further investment or R&D (estimated production costs are €10-20).

Regarding the market size of the animal market, pigs are mainly tested in groups (pooling samples as with PCR). For saliva sample collection, a special rope is used, where a group of pigs chew and leave saliva on it. A PCR test is made, if a positive raises, all pooled animals are tested individually. There is an interest in modeling influenza transmission to prevent zoonotic transmission. Considering that in 2020 the number of pigs in the Netherlands and Germany is about 36 million, if we use 10 pigs as a group to test, then, we need to perform 3.6 million tests, which is a large enough market. The second-stage development market is Germany's nursing homes and hospitals. For human beings, we pay more attention to the individual rather than the collective. Therefore, we focus on vulnerable elderly people in Germany because of the high degree of aging. Germany currently has 18.14 million people above 65 years old, accounting for 21.7% of the total population [14]. The income from the first phase is invested in the second phase of clinical trials. By doing this, we can better control the company, because in this way we can avoid large-scale venture capital, which will cause the founder's equity to be diluted. In the human case, hospitals also suffer from the logistic delay in receiving a PCR test. Patients with potential infectious diseases need to be quarantined for several hours. During interviews with medical clinicians we concluded that hospitals want to know quickly if a patient is infected or not. The cost of the test is justified by the savings in shutdown time and outbreak protocol breach.

6 Team and support (max. 1 A4)

6.1 Contributions of the Team members

Marina Castro Guerrero (Master Nanotechnology, 1st year) is one of the team captains. Her main role was to organize the team and make sure the deliverables and deadlines are reached in time. Besides, she was part of the chemistry and the optical labs working groups.
Luan Duong (Master Biomedical Engineering,1st year) his main role was participating in the optical lab working group and software development. He helped with the testing and optimization of the setup in the optics lab.
Tharanghi Logendran (Master Technical Medicine, 2nd year) her main role was online support. With this role, she took part in interviews and meetings in which she could provide a clinical point of view.
Junhua Luo's (Bachelor International Business Administration, 2nd year) main role was to lead the development of the business plan. He was responsible for the business side of our sensor development and for the market research.

Sharath Rameshbabu (Master Nanotechnology, 1st year) was pivotal in the laboratory testing for both chemistry and optical labs working groups. He participated in developing the surface chemistry for the biosensor and in testing and optimizing the setup in the optics lab.
Sara Schooten (Bachelor Technical Medicine, 3rd year) her main role was to help the optics lab working group. She was also in charge of managing the weekly activities of the team, including minute taking.
Nina van Weperen (Bachelor Health Sciences, 2nd year) is one of the team captains. Her main role was to organize the team and be aware of the deadlines, as well as preparing the agenda for the weekly meetings. She had a supportive role in different working groups, mainly in the business plan and the optics lab working groups.
Sylvia Zijlstra (Bachelor Health Sciences, 2nd year) main role was managing the social networks of the team. She was the team promotion person and was also responsible for the external communication of the team. Besides she contributed in writing the business plan.

6.2 People who have given support

Edwin Beckers, a business manager Medical Systems unit from Demcon, supports us in identifying customer segments and market size.
Floor Rodenberg-Ten Berge, a general practitioner in Kesteren, supports us in understanding influenza symptoms and current methods to detect Influenza virus, mostly in PCR.
Gerdien van Schaik, an Epidemiologist from Royal GD Animal Health, supports us in developing knowledge in animal health and exploring the animal market as an entry market.
Melanie Lindenberg, a MedTech R&D Implementation specialist from TechMed Center University of Twente, supports us in understanding real-life medical device implementation and introducing medical device regulation.
Erhard van der Vries, a senior scientist at Royal GD Animal Health with over 12 year of experience in influenza research, supports us in understanding the current issues in detecting influenza within pigs.
Keshen Mathura, an expert on medical device regulations and health technology implementation from University of Twente, supports us in understanding European Medical Device Regulation and the importance of CE Certificate for the human market.
Marie Weijler, a venture capitalist from Cottonwood Technology Fund, supports us in understanding venture capital's investment strategy, potential investment opportunities in the healthcare sector and developing alternative markets.
Nashwan al Naiemi, a clinical microbiologist from LabMicTA, supports knowledge in influenza in humans and the overview cost of influenza detection.
Anke Huckriede, a virologist and vaccinologist from University Medical Center Groningen, supports knowledge in influenza.

Dr. ing. Josep Canyelles Pericàs has been the daily supervisor from the team. He helped with organizational matters, and he was also the linking person with MESA+.
prof.dr.ir. Loes I. Segerink helped with bureaucracy matters and was also the link between the team and the University.
Ir. Raimond N. Frentrop supervised the photonic biosensing experiments in the optics lab and helped the team with his expertise. He also helped with the setup building and testing.
Dr. ir. Nico J. Overeem supervised the surface chemistry processes and also helped the team with his expertise. He helped with the development of the antifouling layer and amplification.
Prof. dr. Sonia M. García Blanco is the principal investigator of the Optical Science group. She helped with resources (in the optics lab) and advised the team with her expertise in photonic biosensors.

7.3 Sponsors

MESA+ is the main sponsor of the team. MESA+ supports the team financially.
Micronit supported the team with expertise. They helped the team with knowledge about the microfluidics of the setup and the pumping system.
Demcon supported the team with expertise. They helped the team with the business plan and market research. They also helped with knowledge about miniaturization and integration of different components in the setup.
Bronkhorst also supported the team with expertise. They helped the team with knowledge about flow control systems.
3T supported the team with their expertise in electronic instrumentation.

7 Final Remarks (max. ½ A4)

This document is truthfully signed by:
Team Captains:
Nina van Weperen: Marina Castro Guerrero:

Supervisor:

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9 Appendix

Appendix A: Main script for data analysis

```
%% BAREBONE SCRIPT for data analysis
% This script from the MATLAB file contains the steps to
%   analyze and process the data from the sensor to understandable graphs.
% Input: all the measurement files, and the desired ring to be analyzed.
% 1st - The data is firstly sketched so
%   the users can choose one suitable peak (min_lambda and max_lambda).
% 2nd - This peak is then fitted with a Lorentz curve to find
%   the exact location of the tip.
% 3rd - Repeated with all the measuremnts

clear all ; close all
% Choose the data - CHECK THE INPUT FILE/ NOT AUTOMATIC
no=474; % number of measurement
ring=3; % chosing the ring

% Start loop for muliple measurements
for i=1:no % Or choose i to see 1 experiment
% Import the data
n_strPadded = sprintf('%03d', i );
data=importdata(['measurement_',n_strPadded,'.txt']);
chosen_data=data(:,ring+1);

% Sketch the measurement data
max_range=1045; % Given from set-up
min_range=1035; % Given from set-up
dt=(max_range-min_range)/length(chosen_data);
t=min_range:dt:max_range-dt; % Wavelength range
figure(1)
plot(t,chosen_data)
title('Measurement result')
xlabel('Wavelength')
ylabel('Intensity')

% Select a peak
min_lambda=1037.8; % choose it from the graph above
max_lambda=1038.3; % choose it from the graph above
ind=find(t>min_lambda&t<max_lambda);
t_restricted=t(ind); % restrict the data to data around the peak
chosen_data_restricted=chosen_data(ind);
```

```
tip_ind=find(chosen_data_restricted==max(chosen_data_restricted)); %the peak
t_tip=find(t==t_restricted(tip_ind(1))); % the peak
limit=3000; % choose 3000 steps around the peak
t=t(t_tip-limit:t_tip+limit);
chosen_data=chosen_data(t_tip-limit:t_tip+limit);

% Fitting the data with Lorentz curve
mindata=min(chosen_data);
data_lorentz_zero=chosen_data+abs(mindata); % plus the base to make it 0
[YPRIME,PARAMS,RESNORM,RESIDUAL,JACOBIAN]=lorentzfit(t,data_lorentz_zero');

% Location of the peak
ind=find(YPRIME==max(YPRIME));
peak(i)=t(ind);

% Plot the fitting
figure(2)
hold on
plot(t,chosen_data)
plot(t,YPRIME)
xline(t(ind),'-','Peak Location')
legend('Measurement data', 'Fitting data','Peak Location')
title('Selected peak and its fitting')
xlabel('Wavelength')
ylabel('Intensity')

% Showing which loop
i
end

figure(4)
title('The location of the peak');
plot(0:10.8:10.8*(length(peak)-1),medfilt1(peak))
title('The location of the peak');
xlabel('Time(s)');
ylabel('Wavelength')
```

Appendix B: user interface

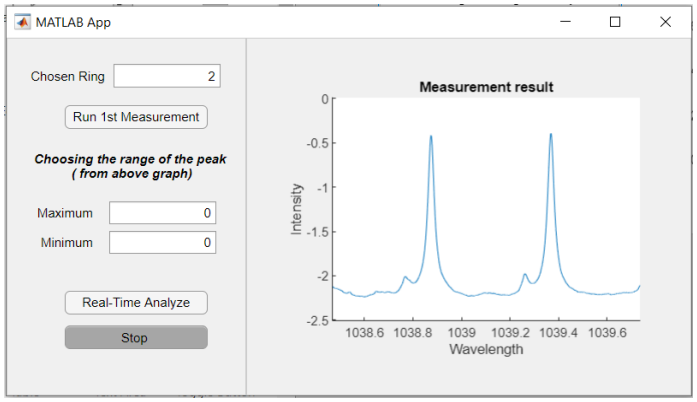


Fig. 6 User interface

For the data analysis, a custom-written MATLAB script is used. The script reads the data from the data acquisition system, which collects the spectra of the output signal (which contains a peak for every resonant wavelength) as can be seen from the Fig. 2a. Then, it selects one peak and finds the exact location of this peak

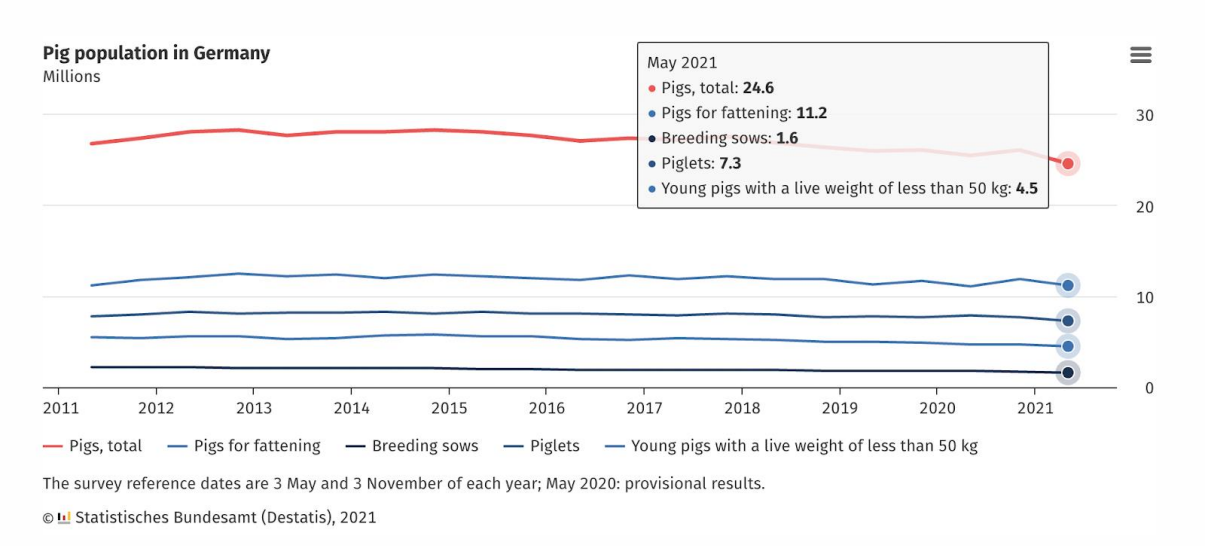
by fitting the Lorentz curve (Fig. 2b). The process is repeated through all the measurements, during which the location in wavelength of this specific peak is graphed. In this graph the wavelength peaks (y axis) are plotted against the time (x axis), of which an example is shown in Fig. 5. Due to fitting errors or experiment noise (such as bubbles, vibration etc.), outfitting might happen through this process. In case of out-fitting, the outliers are filtered out of the desired data using a third-order one-dimensional median filter. Using a calibration curve (which is measured beforehand), the wavelength shift is correlated to the different concentrations of the analyte so that the unknown concentration of the analyte can be calculated from a measured wavelength shift. These calculations can be found in the script in the appendix A.
The user interface is built using the MATLAB App function (Fig. 6), which is based on the MATLAB script developed above. The interface simplifies handling of the code and helps users to smoothly analyze the data. Regarding the user handling, the only thing that needs to be done is place the chip on the chip holder. The alignment will be done automatically. Then, the sample should be placed in one of the flow inlets. Finally, the system can be turned on and the software can be runned. Real-time analyzing of the data can be carried out until the experiment stops. The application will return the wavelength-shifted plot and automatically calculate the analyte concentration to such an extent that no other handling from the user is required.

Appendix C: Translation potential

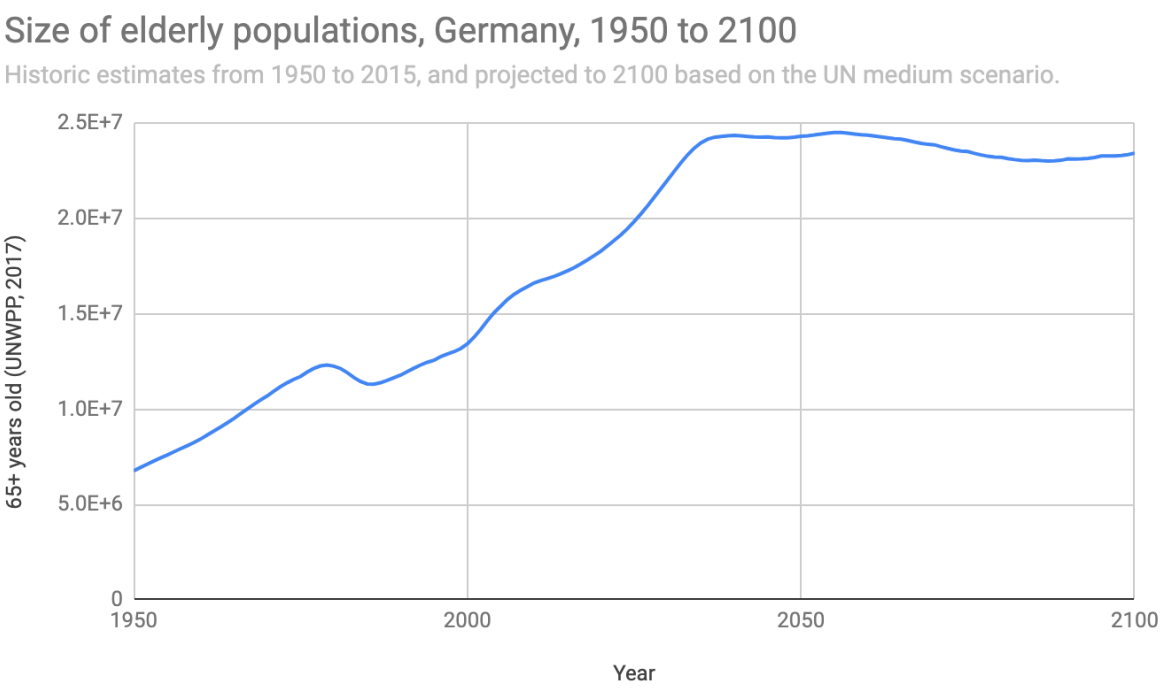
Number of pigs and pig farms in the Netherlands [19]

The Netherlands	**Housed animals**	
	Number of animals	Number of farms
	Pigs	Pigs
	Pigs, total	Pigs, total
	number	
2000	13,117,814	14,523
2005	11,311,558	9,686
2010	12,254,972	7,030
2015	12,602,888	4,928
2016	12,478,594	4,508
2017	12,400,699	4,301
2018	12,430,129	4,185
2019	12,269,154	4,087
2020	11,950,238	3,557

Number of Pigs in Germany [13]



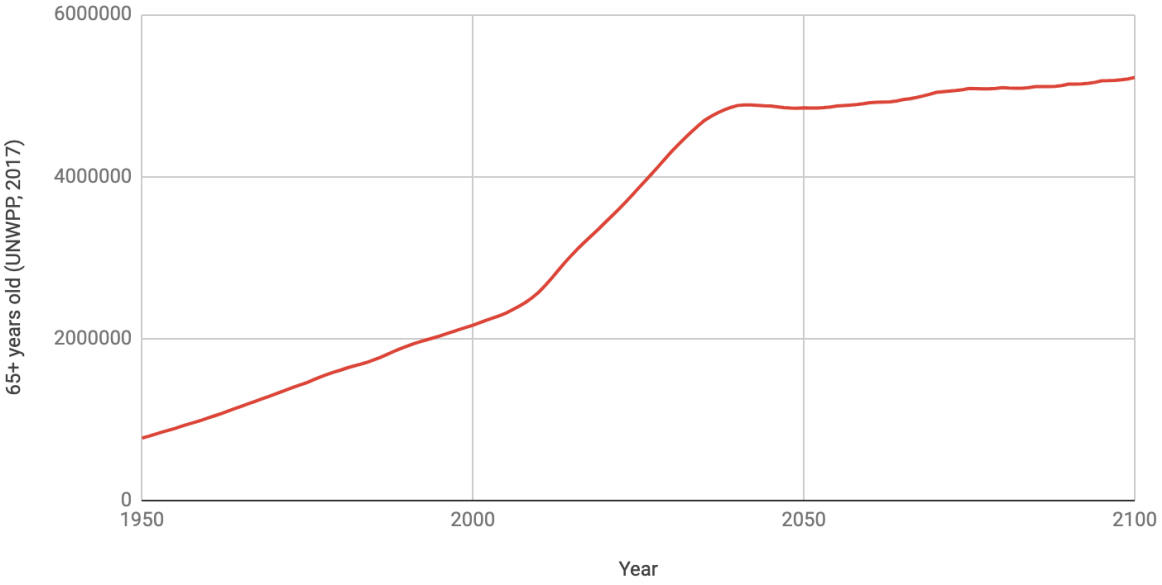
Size of elderly populations, Germany, 1950 to 2100 [14]



Size of elderly populations, Netherlands, 1950 to 2100 [14]

Size of elderly populations, the Netherlands, 1950 to 2100

Historic estimates from 1950 to 2015, and projected to 2100 based on the UN medium scenario.



Appendix D: Feedback collection interview transcripts

All the transcripts below were read and approved for publication by the subjects.

1. Interview Royal GD Animal Health - Gerdien van Schaik

Date: 03-06-2021
Present: Gerdien van Schaik (Royal GD Animal Health), Pep Canyelles Pericas, Nico Overeem, Tharaghi Logendran, Junhua Luo
Minutes: Junhua v1.0. Revised: Pep

Background: Royal GD is focused on animal health. They run a large monitoring and surveillance system, commissioned by the government and industry. Gerdien is the head of the epidemiology group, designing all kinds of projects. Mainly focused on farm animals with monitoring and control of diseases. She is also a professor at Utrecht University (professor in monitoring and surveillance of farm animal health).

Summary:
Gerdien first introduces how to detect the influenza within animals and points out an example of detecting influenza in pigs. The cost to detect influenza in pigs is 40-50 euro (max). It is done with PCR and with sending the sample to a central lab. This takes about 24h to get a result. Sample is collected combining ropes (pooling) and pigs chewing to it and leaving saliva. Then the sample is collected and tested. If positive it traces back to the group of animals that provided the sample. Actually there is not much information about influenza transmission in pigs. As this disease has zoonotic potential (risk) it is interesting to obtain more information about that. In fact this is one of the diseases that GD is looking into because of that. Market size: Around 2000 herds in the Netherlands would purchase the diagnostics. Slaughterhouse, 12 millions pigs/ year. 100 millions chickens. There is also influenza for chickens but they don't have saliva for sample collection. Chicken are tested individually for influenza. Another option could be test uses as a sample the environment that the chickens are in contact with. For instance the dust in the chicken barn. Every 6-8 weeks the cycle is completed and animals are sent to the slaughter house. Before putting new chicks in, the barns are cleaned and tested for diseases. In poultry the price per test is lower than pigs.

They usually use an indirect way of testing - detecting the environment, and samples will be collected as a pool (in animals tests are made in groups, as opposed to humans where tests are individual). When detecting animals, they are more curious about the group, not the individuals. They only care whether the virus circulates within the animals. When animals are ill, vaccinating animals is an option instead of sacrificing them.

Question: What is the preferred type of detection?
Answer: The validity of the test. It depends on how you deal with the result. High specificity, high sensitivity. The size of the population.

Question:What factors could make tests more attractive?
Answer: She believes it should be similar to the current detection method so they could prevent the spread and avoid loss. Taking samples from saliva is a good option because drawing blood is painful for animals. Another factor they take into account is the validity of the result (specificity, sensitivity and accuracy). Logistics in PCR are also a problem. Sample is collected, sent to a lab and a result is given 24h. If there is a positive it might be difficult to trace the animals back. On site testing with electronic storage and transmission capabilities would be ideal.

Question: What makes them turn to use new devices?
Answer: For animals, they consider the technological validity (specificity, sensitivity). Can we put a lower cost or make a higher margin? How can we deal with it in the lab? What would the investment be? And can we earn from it? In animal health cost is a major driving force, more than with humans. Technical validity (better test) is also important but secondary to that. Tests that can be run in the current labs (or without lab) with no investment needed are also very appealing.

Two reasons for GD developing own products and perform the test at lab:
1. The validity of the result. In the lab, GD will do it the right way.
2. They are concerned with data kept by the organization. If they can't perform the result in the lab they will lose the data. Having a test on site that transmits the data electronically directly is also very interesting. If such a test would be widely adopted GD would have a big picture on influenza transmission.

She suggests we find Influenza -related data from RIVM(human Influenza), CBS(production data, farms, animals) as well as Google flu model(Not sure if it still exists). However, the data for Influenza can not represent the whole situation because the data only will be reported if the patients visit the general practitioner (for instance, people do the self test at home during the covid-19 situation). The corona-19 situation also leads to the missing data of Influenza this year. It follows the previous point: modelling Influenza transmission with humans is important. Tests with electronic data transmission to government databases would be ideal.

The disadvantage of PCR:
It is an expensive test (just the antibody usually costs 8 euro) due to all the materials, labours. All in, it costs 40-50 euros and it takes 24h to get the result. They will usually get the result of PCR the next day. Besides, different samples have different requirements to keep. They also need to think about taking samples to the lab (logistic issues such as van fleet, drivers, lab personnel). Data is not centralised, and this is an issue for modelling infectious disease transmission.
The advantage of PCR:
A lot of experience with the test, very specific, multiplexing capabilities.

2. Interview Royal GD Animal Health - Erhard van der Vries

Date: 18-06-2021
Present: Erhard van der Vries (Royal GD Animal Health), Nico Overeem, Marina Castro Guerrero, Sharath Rameshbabu, Pep Canyelles Pericas, Nina van Weperen
Minutes: Nina v1.0, rev. Pep

Background
Erhard is senior scientist at GD animal health with over 12 year of experience in Influenza . He is interested in how to detect Influenza in animals.

Summary
He thinks Influenza is the most important virus in the world, but the pig market doesn't see it like that. They see this PRRS virus as the most important virus. PRRSV is a respiratory virus and also a reproductive virus. It affects the macrophages in the lunges. From there it goes into the blood, you can detect it in serum. PRRSV is pretty similar to Influenza . The way industry deals with this problem is with vaccinations. However the vaccinations are an "attenuated" version of the virus, which also comes up in tests. This creates a big problem. If your setup could detect and differentiate both vaccine and wild virus, it would be a breakthrough (a "holy grail" in his words). Can you also detect other viruses with the same technology?

If you want to test Influenza , you can start with the pig market and continue with humans.
This is a big market, a big industry with a lot of money, so if you think of an entry market with a lot of money, it's the pig market. For them Influenza is the second target, and PRRSV the first target. This is because it costs billions to the industry.
But there are more animals in the Netherlands than humans, so it could be an interesting entry market. The pig market in the Netherlands is huge.

Influenza comes from animals. You could ask yourselves, what is circulating in animals that could potentially harm humans? The zoonotic potential of Influenza makes your technology more attractive (PRRSV is not zoonotic, so no threat to human transmission). Following this lead you could ask yourselves about diseases that could be transmitted from animals to humans.

There are all kinds of regulations when it comes to exporting and importing livestock. The regulations regarding PRRSV is that if you want to export pigs to, let's say Spain, then you need to ensure that the pigs that you are exporting are PRRSV free.
This is a problem because: the big issue within the pig market is that you get the spread of the PRRSV vaccine and the PRRS virus, and they want to distinguish the vaccine virus and the field virus, but that is not possible yet. If you could make an essay that could do that, then you have a high potential in the pig industry.

Erhard is not sure if Influenza alone is the best business case for the pig market. Then you probably have to combine it with PRRSV. If we add something with PRRSV on it, that would be more sellable.

Many people focus on humans, so maybe it's interesting to focus on animals, it's a different point of view. If you think of a platform that needs to make money as a business case, and you need an entry market, then you can consider the PRRSV virus and the pig market.

It's also good to think of the sampling method. The traditional PCR is relying on conventional ways (long paths) of diagnostics. In this pandemic, it wasn't a good way to handle such huge volumes of data, it's a huge logistical problem. If you could go for an alternative logistic route, if you detect it on the spot, and the result goes via the internet to the doctor, that is a big progress. That's also an advantage of your chip.

For a company like GD, it's also important to have the data. Generally hospitals and companies want to have the data, not only the rapid tests. If there is a way to connect end results to data, that would be great. The data is the potential of the business. Companies like GD do want this data. If you can capture the data, and make it useful, for surveillance and monitoring of diseases, that's the real add on.

The corona PCR, they gave a price of 100 euros, that's the price they normally ask at an academic hospital. But now it's sold for much less, and the clinical doctors weren't happy with that. So because of the pandemic, big insurers companies are now very much aware of the real costs of a PCR test. So the clinical doctors are never able to sell the pcr tests for 100 euros anymore. After the pandemic there will be a real fight on diagnostic prices.

Is there a difference between a nose swab or saliva in Influenza ?
Everybody probably had this nose test, it scraps a bit of cells into this swab.
Especially in PCR tests, where you are searching for RNA of the virus, I think that could be important. If you have some infected cells, they are full of RNA, if you have them on your swab, that's an advantage, and makes it more sensitive.
I'm not sure about RNA levels in saliva, so that's difficult to compare. But there may be relatively more viral proteins compared to the RNA in saliva. That could be.
This discussion related to the logistics: when sending a sample for 12h in room temperature the virus that you want to detect degrades. Testing on the spot would eliminate this problem (which we can do).
I'm not sure about the proteases in saliva, how they influence your sensitivity and also how long your sample is good enough to test.

3. Interview TechMed Center - Melanie Lindenberg

Date: 10-06-2021
Present: Melanie Lindenberg (TechMed Center), Pep Canyelles Pericas, Junhua Luo

1. How to determine the market?
   She suggests that we should start with the population. If the business model has a specific target on hand for dianogist device then it is wise to start with statistics on how many people we have yearly to use that device in the Netherlands or in the world. Try to make an assumption of how many percent of the population will definitely use the diagnosis device and who is coming to that place to be clear about the size of the market. Evaluate the market size in five year beyond the market and it is always wise to look at the trend of population that is varying over years whether you can explain the next years and the expected market when your device is ready. Make situations as well as make the modelling and scenarios analysis (positive and negative).

2. How can we translate scenarios into one business model?
She suggests we should calculate both situations and think about how they might impact other strategies, for instance, market strategies. Not only focus on the population that the device you will sell, and other things you can imagine which will have impact on and spend one page describing two scenarios and combine them into your business plan and take some time to think about a bit more either the pandemic or the non-pandemic one.

3. What is the reason for hospitals to use a new device when they already have one?
One main aspect is logistics, we focus on detection settings for Influenza A and B and they have points of general test PCR, CPR. We look into why we use this test? In this case, they don't need to send samples to the lab if there is an urgent situation in the lab. There is the benefit they saw for point of view. Another thing they consider is the money, you have to show the device you developed is less expensive and more accurate. If you develop a new device, it should be usable and have a walk through to see how technology currently works and make sure you are able to speak to the end-user.

4. Is there any regulation that will influence the device launching?
It is a recent topic the MDR (The medical device regulation) has been implementing since 26 of May. There was some European guidance to follow but you are not restricted to that and now it becomes a law. All diagnosis devices, software devices, and artificial intelligence have to fulfill specific requirements. MDR is different per country.

4. Interview Demcon - Edwin Beckers

Date: 11-06-2021
Present: Edwin Beckers (Demcon), Junhua Luo, Marina Castro Guerrero, Sylvia Zijlstra

1. **Who do we think is the customer?**
How insurance works: Companies don't care about how fancy it is, so it should be cheaper and better to whatever is now in the market. In the hospital they don't care about the costs, they only care about the performance, so good accuracy and an efficient process. But if you ask them they will probably don't want any change.

   Likely people from the hospital don't care about if they have 10 machines because they have space and money. They wouldn't want to buy new equipment. You have to target the novelty or the real problem they have with current methods. Making it smaller or cheaper is not an added value for people that work in a hospital.
   Devil's triangle: quality, time, cost
   Both parties are interested in different parts of this triangle. In the case of hospitals it's special, you have to come up with specific needs. For example Influenza would not require faster tests (because in hospitals they don't care about it).
   Person paying, person using, person getting the results of the device.
   The most important part of the devil's triangle strongly depends on who you are targeting. We need to find the balance.
   It's called willingness to pay, and the sudden need.

2. **How to identify the market size:**
Google is very useful for it. You have the bcc research for it in a report, they give pieces of the information and you can afterwards buy the report if you need (but we don't). We can just do some research and figure the global numbers for the market size. What do they take into account for the calculation of market size? Only biosensing? Also the syringe to extract samples?, etc.

Investors will ask which is our first target market so we need to figure out.
Even different countries will have different regulations which will make our business case different. We should know the customer well.
Also depending on the country it is better to target customers instead of hospitals, as the healthcare is private so it's the customers who will pay for it so decide to which hospital they want to go.
Depending on the target market there are different problems that we'll face so we should take that into consideration (for example, if we need to do marketing, or if we target a developing country).
Key opinion leader from a company is one of the most important people to talk to.

5. Interview Cottonwood - Marie Weijler

Date: 18-06-2021
Present: Marie Weijler (Cottonwood), Junhua Luo, Marina Castro Guerrero, Pep Canyelles Pericas

1. **As a venture capitalist, how do you select a good project? Any criteria?**
   Each venture capital firm has its own criteria. Cottonwood invests in hardware and high tech and only invests in patent based technology. Software is difficult to patent so VCs that invest in software don't have this criteria. In the case of cottonwood, they only invest in very novel projects. Regarding the team, they look at the co-founders and decide whether they will be able to work together or not (the chemistry between them). In their case, they invest in team players, someone who has nice team work skills.

2. **How do we deal with conflict between founder and investor?**
   If it's a problem that can be solved, they will. If it's a personality matter or values matter they will not invest.

3. **Is it the Covid-19 situation increasing the possibility to invest in the healthcare sector? Is it a high potential market?**
   Definitely. Even before Covid-19, healthcare was very popular within investors. It's always a growing market. At first with coronavirus, some investors stopped to see what was happening but now they are back on track and the investments have increased (regarding monitoring, diagnoses, etc.). In their case, they only invest in projects that can be used in several different ways. For example, the patch/chip to check how the lung works is interesting because it can be used for Covid-19, asthma, athletes, etc. But it depends on the specific case.Investing in a product that has different applications decreases the risk of the investment for the VCs.

4. **How to deal with the uncertainty in the future?**
   Uncertainty is something that VCs have to live with. There are different strategies to de-risk. For example, the different applications. We should analyse how our technology can be used if there is no pandemic. Licensing is an option, also pandemic prevention. We could also look for other target markets.

5. **Influenza**
   Influenza will always be there so it's a big market. Even if Covid19 gets behind this, people will want to prevent the next pandemic. If this is the way to prevent it, it will be a nice investment. We should check if we make something cheaper or better than competitors. We should always be clear and open about competitors. Just Don't lie about competitors. Don't be pretentious. Don't compare yourself to someone else and don't be afraid to admit some skills are missing in the team.

6. **Right now, the Influenza market in the Netherlands seems pretty small so we find it difficult to translate into a business plan. What would you recommend for the translation into a business plan?**

The market will be small anyway because The Netherlands is a small country. As a recommendation, look into Germany. It's a big, strong country and the culture is similar. Also if Germany would want the solution, Scandinavian people would be interested in it. And if many countries in Europe are interested in it, probably the USA and China will also be. Also France is a good country to look into. Korea, Singapore are also interesting. Skip Africa.
As an advice, take advantage of the different cultural backgrounds in the team (for example, Junhua can look into China for the market search).
If a startup team can show they worked together before in a different environment it's a strong case as it proves they can work together well.

6. Interview Medical Device Regulations - Keshen Mathura

Date/Time: 11.00 AM - 12.00 AM, 21/06/21
Present: Keshen Matura, Pep Canyelles Pericas, Sylvia, Nina
Minutes: Sylvia (v1.0), revised Pep

Background
Keshen is a lecturer on Health Technology Implementation on BSc Health Sciences. Sylvia asked Keshen if he could provide some feedback on our views regarding medical regulations. Keshen Matura has been at the UT only for a few months. Previous to that he was based in Amsterdam, where he did work with startups in the medical device domain. He is an expert in clinical and regulatory landscaping for medical device development. At the UT he is teaching modules related to this subject and helping UT researchers and students to navigate the regulatory side in clinical translation.

Questions

1. Do we need CE to put it to market? If we start with research use only then we don't need CE marking. Is this correct?
    - Yes, this is correct. The device would be a class C in vitro (our device has this category because it is not attached to the patient and the sample is collected non-invasively). To achieve this certification certain rules and regulations must be followed. Certain rules and regulations need to be followed.
    - Validation of measurement (testing methodology & measurement technology?)
    - Risk analysis (different perspectives on what could go wrong with the product, i.e. light, antibodies, electronics, etc.). He has a lecture specific to this topic in the Health Science course. Risk analysis needs to be incorporated in the development.
        - User perspective
        - Clinical perspective
        - Light
        - Antibodies
        - Minimal specificity & sensitivity (this is key)
        - How to address those risks?
    - How you are going to validate
        - Are the risks appropriately covered?
    - Clinical evaluation
        - All similar technologies on the market (analyse them)
        - How long has it been on the market? Is there any literature that provides us with additional information (patents, papers)?
        - Database, adverse events
        - Establish a base of knowledge in the company (using the points above)
        - Any similar technologies, we identified and put it in the risk analysis.
    - Pre clinical evaluation and testing
        - Not in humans (contact) but sample coming from human materials (saliva). Could blood phantoms be used for testing?

    - Sensitivity test: 100 tests, write the report in paper style: introduction, methods and materials, results, conclusions.
    - Once this is all established, define a bill of materials (supplied from? Pay attention to suppliers, parts might need to be medically certified). You can do external testing for electronic compliance and radiation.
    - With everything before, now we are going to do lab tests, not on humans.
    - Everything must been proved
    - We have to prove the sensitivity etc.
    - We have gathered all types of risks
    - Different test under different circumstances
- Once all that is done, then you can set up anything else.
- During the research, you don't need a CE (investigational device) (for research use only). It is possible to start with 'research use only' market, as it does not require certification or trials. The numbers are smaller (research use only), but application data (papers) resulting from device use are valuable for validation (and to show that your stuff works).

2. What do you think of our Market Feasibility: Does anyone want it?
    - Productivity loss with Influenza (find some stats from government databases)
    - Deaths (Influenza mortality, possibly affects the elderly predominantly, but effect in the workforce? As it is important for the economy).
    - What are the rates of Influenza worldwide?
    - If you have a rapid detection method that people can do at home. Promoted at Etos, Kruidvat to stay home if the test is positive. You have to give a message with the test, you can't just let them test themselves, and then they have just the results. The question is: what should they do with the results? If there are no consequenties to the detection it will not be attractive, so what is the consequence of the detection?
    - A question for you is what are you going to do with a positive test? Are you going to provide treatment? Ask people to call GGD and stay at home? But some people might not do it. The device could transmit the positive to GGD directly with embedded electronic capabilities. Governments are concerned with data and obtaining an accurate picture of flu transmission.
    - Flu vaccines are not 100% effective (only 60-70%), which creates a market for the elderly population (i.e. detection and isolation of Influenza cases in care homes). Same applies for hospitals. Big savings can be deducted from fast detection and swift isolation as it stops spreading Influenza within the hospital. If you get some numbers on Influenza deaths in hospitals you can construct a story around this. Similarly a device could be used by a GP to screen elderly people. What would be the cost of having a device in a GP practice?
    - Benchmark performance with PCR (as golden standard method). Cost reduction is a good argument for insurance companies and governments.
    - Can the product be expanded to the western world (USA, Canada, Australia, Japan)? After expanding to these markets the device becomes more visible and it is likely that the World Health Organisation regulates it, which opens the door to Africa and Asia.
    - Market research in EU/US: Eurostat (a lot of data, open and free). German government statistics have a tremendous level of detail and are also open. In the US, use the Centre for Disease Control (CDC), which also has detailed statistics.
    - Find reports from Influenza made by the WHO.
    - There is a lot of research (review papers) on demographics that can be used to model the market. Compile the info from the previous bullet points in an Excel and add as appendix to the business plan.
    - Market size: start with Germany (big market, gateway to EU and then US). Research use only for Germany is good, they like that. Many NL startups start with Germany. It validates the performance in a big market. VCs like that. And there are a lot of elderly people in Germany, Italy and France. The Netherlands and Scandinavia are not good entry markets because of smaller populations.
    - Make an evidence based business plan, backed by reports and papers.
    - One part avoiding deaths
    - Elderly dying from an economic perspective is not that important.

- Easier + from home can stem (indammen) de virus
- Infection in hospitals from Influenza → Can save costs.

  Questions we need to answer:
- How many infections of Influenza happen in the hospital?
- Infection in hospitals from Influenza

3. Financial Feasibility (cost of medical device regulation)
   - Commercial costs (if outsourced): 100,00 - 200,000 euros
   - Commercial costs (if developed inhouse): 60,000 - 80,000 euros
   - CE certification = 20,000 - 60,000 euros (for your device probably 30k - 40k)
   - Time scales: 9/12 months, 6 months at minimum (for CE certification)
   - Timescales to reach the market: 6 months for pre-clinicial evaluation, 6 months for clinical testing, 6 months to a year for CE certification (estimated 2 years to reach the market, min 100k investment, could easily be 250k)
   - It is possible to avoid CE marking if it is an investigational device (research use only), but the market is then smaller.

4. How to target the hospital?
   - Bijv zorggroep voor meerdere elderly homes
   - Do research in EU and US (a lot of statistics)
   - WHO
   - German statistics department (very detailed)
   - PubMed
   - Mortality rates of Influenza in different groups
   - Netherlands is maybe too small to start -> Germany way bigger market, more easy access. Most startups take Germany as first market (considerable size, gateway to rest of EU and US)
   - If it is going well in germany, almost guaranteed you do well in the entire EU
   - Keep it all evidence-based instead of information-based
   - You can put all the numbers of Influenza per country in an excel sheet(the numbers of the countries) and add it in the business plan. This way you can show how you decided your customer.

5. Technical development
   - Identifying compatibility of materials (issues with plastic) and light specificity effects, might play a role in the detection performance.
   - Distinguish vital parts and product design (user centered).