DeTectUs Team
Technical University of Denmark

SensUs Competition 2021

Team Results Document

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1 Summary

Our team decided to use a paper based diagnostics approach where a Lateral Flow Assay was performed. By immobilizing one type of antibodies to the nitrocellulose membrane and conjugating another antibody to gold nanoparticles (AuNP), we achieve the detection of the H1 protein specifically in the saliva sample. The agglutination of the two antibodies to the antigen together with the AuNP allows for the detection of a colorimetric signal in the membrane. We developed and assembled a reading device where our cartridge is inserted and the saliva sample is added to start the process. The device can record the membrane and measure the change in color intensity over time. Then, through a linear regression model, it predicts the antigen concentration in the analyzed sample. The target market chosen focuses on veterinary applications in livestock production. Influenza detection, along with other diseases in pig farms, is an essential step to meet Sustainable Development Goal number 12 for sustainable production and EU regulations, and therefore this market is in need of a portable and quick solution.

2 Biosensor system and assay

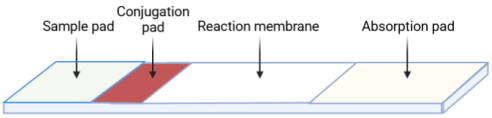
2.1 Molecular recognition and assay reagents

Antibodies (Abs) are specialized Y-shaped proteins produced by plasma cells in response to an antigen. Antigens (Ag) can be anything that causes the body to make a specific immune response, such as viruses. Each Ab is specific to one Ag. One of the common surface Ags recognized by Abs in an Influenza infection is hemagglutinin (HA)(1). Most Ab responses against HA are strain-specific, with H1 being an Ag characteristic for Influenza A. Due to low availability of attenuated Influenza virus particles, in this year's project we have targeted the H1 particles only (2)(3).
As antibody and antigen react both proteins combine in an agglutination process. There are two types of antibodies depending on their specificity: monoclonal or polyclonal. Monoclonal Ab recognize only a specific region of the Ag structure, having therefore monovalent affinity, polyclonal Abs bind to different regions of the Ag (1). Our lateral flow assay utilizes immobilized capture antibodies (cAb) on a nitrocellulose reaction membrane together with a detection antibody (dAb) conjugated with gold nanoparticles (AuNPs), giving a dark red color.

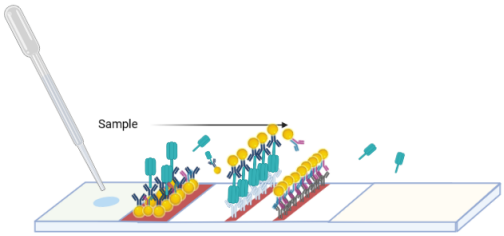
Our chosen antibodies are a monoclonal mouse antibody IgG2b (Hemagglutinin-HA-11055-MM08) that detects hemagglutinin from the H1N1 Influenza A strain and a polyclonal IgG (Hemagglutinin-HA-11683-T54) that has been functionalized with AuNPs for the detection.

2.2 Physical transduction

The physical sensing principle is based on two main elements: The LFA strip and an optical signal. Our Lateral Flow Assay (LFA) strips are composed of: A sample pad, a conjugation pad in which AuNPs conjugated with the dAb are embedded, the reaction membrane containing dots functionalized with cAb and control antibodies (secondary antibodies that capture the detection antibody) and an absorption pad which drives the flow through capillary action (see Fig.1a)(4).
The optical signal is created by the AuNPs. As liquid migrates from the sample pad to the reaction membrane via the conjugation pad, the Ag, if present, binds to the dAb, and drags the dAb-AuNP molecules towards the reaction membrane. Once the complex, Ag-dAb-AuNP, reaches the test dot of the reaction membrane, the immobilized cAbs bind to the same Ag, creating a sandwich-like structure. The dAb also reacts with the control Ab on the control dot. In the absence of analyte, the dAb reacts only with the control Ab. The optical signal forms due to the accumulation of gold nanoparticles due to plasmon resonances(4). This interaction is heavily dependent on the size of AuNPs, which was found to be optimal at 40 nm.



(a) Setup of the LFA.

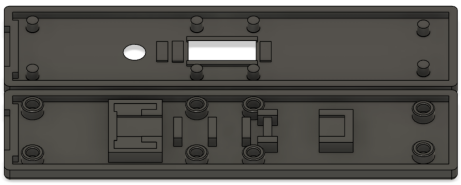


(b) AuNP-dAb-H1 complex captured by cAbs on test line.

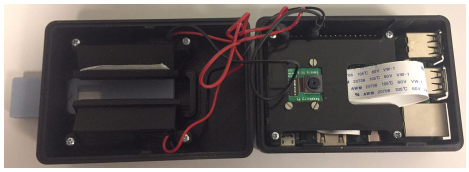
Figure 1: Scheme of assay in the presence of analyte.

2.3 Cartridge technology

Our cartridge is a cost-efficient, 3D printed resin housing that encloses the LFA and fits within the developed reading instrument. The cartridge provides protection to the LFA and stabilization of the fluid flow(5). It consists of an upper and bottom part held together with eight pins in the top part and corresponding pinholes in the bottom.
A sample inlet and readout window are placed over respectively the sample pad and reaction membrane.To ensure homogeneous fluid flow through the LFA, pressure ridges situated over each overlap of components in the LFA are incorporated in the top part.



(a) Top and bottom of the cartridge.



(b) Open device

Figure 2: (a) 3D rendering of the cartridge. (b) Image of Open device

2.4 Reader instrument and user interaction

The reader consists in a Raspberry Pi and a Raspberry Pi camera board combined with a 3D printed box with internal LED illumination (view Fig. 2). The reader acts as a server offering a phone app which allows a readout after colorimetric detection. This detection starts after a cartridge has been inserted where the camera is utilized to capture a movie of the optical signal. By applying processing the resulting temporal signal a concentration can be determined, this is specifically using calibration curves in python.

3 Technological feasibility

In this section, the performance of our developed LFA sensor, along with the remaining challenges and unknowns, is discussed. The performance is evaluated in accordance to the required capabilities of the competition.
Disclaimer: during the project large delays in the supply chain was experienced (due to Covid19 outbreaks). This resulted in inadequate amount calibration tests needed to evaluate the colorimetric detection / algorithm's accuracy, several tests have been made and more are planned to do this evaluation.

3.1 Assay

The LFA sensor is evaluated on three main parameters; time, accuracy and sample handling. The feasibility of the LFA to accommodate the required analytical performance is presented.

3.1.1 Time

The time is of great importance as a rapid diagnostic tool is capable of screening a large crowd whereas a non-rapid sensor would result in long queues and waiting time which is an undesired situation. The time of the LFA was evaluated by measuring the time it takes for a optical signal to appear on the strip. The time begins when the saliva sample is deposited on the LFA strip through the cartridge inlet. The sensor is expected to give a concentration result within 5 minutes. At all concentrations a visible optical signal was detected within 5 minutes. This can however be improved by the data analysis by considering temporal information, this has due to supply chain difficulties not been determined yet.

3.1.2 Accuracy

The accuracy of the LFA was determined by evaluating the sensors ability to measure a range of concentrations given, 1-10,000 pg/mL of H1. The accuracy was also evaluated by determining the sensors performance in determining the concentrations within the concentration range. The sensor is able to detect an optical signal for the entire concentration range from 1-10,000 pg/mL. The ability to determine a concentration from the optical signal was not determined due to supply chain difficulties.

3.1.3 Sample handling

The LFA sensor requires minimal sample handling due to no pretreatment and no dilution required. The saliva sample is taken directly from the sample vial and 30 µL is transferred into the cartridge inlet. The sample volume was decided after optimisation of the components of the LFA strip.

3.2 Device

Image information for each frame in the video was extracted and the frames were trimmed to the size of the membrane strip. By creating a mask using region of interest analysis the color of the optical signal can be extracted (Fig. 3). The pixel values here are then extracted in python after which the calibration curves can be utilized to determine the viral concentration.

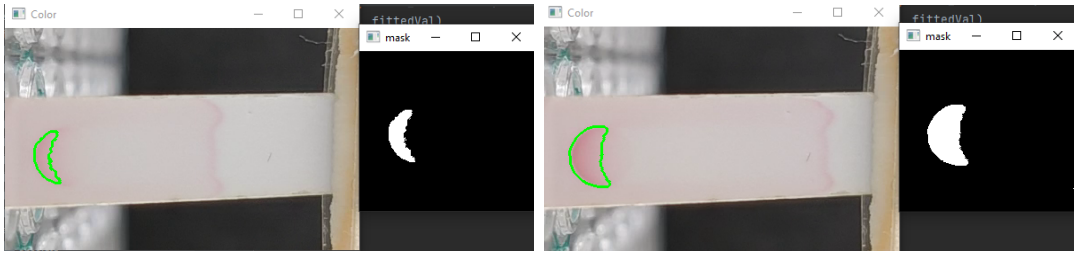


Figure 3: Video frames with the detected contour in green, and the mask detecting the area inside the contour, which is where the color is going to be measured.

To reduce the noise of measurement between frames a curve through the frames was fitted to estimate the concentration and curve parameters. In Fig. 4 it is possible to see the raw data of the intensity measured throughout the video and how it shows a logarithmic growth, and in yellow we can observe the fitted curve.

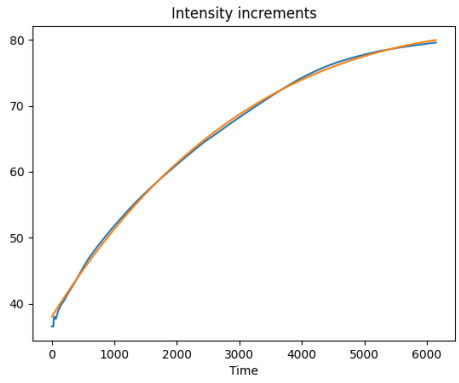


Figure 4: Curve of the color intensity through time (in frame units). Raw data in blue, fitted line in yellow.

For colorimetric detection a linear regression model was used. It receives the parameters estimated from the curve fitting as input, since for each antigen concentration the dynamic change of color intensity is different, and the prediction of the antigen concentration as output.

4 Originality

Team Captains Our goal was to develop a rapid and user-friendly PoC device. Our concept consists of different components that interact to make a ready-to-use product. We developed an effective analyte recognition framework through careful experimentation with the immunoreagents. We optimized the geometry of the LFA and its time-efficiency integrating the pumping-free LFA strip with a functional cartridge. We implemented a device for image acquisition and an algorithm for dynamic prediction of the protein concentration. The reading device is the core element of our innovation, as the optimal lighting, the imaging and the dynamic prediction algorithm allows reduction of the response time independently of the sensor. We approached the problem posed by the SensUs competition so that we could develop a complete product. This was also achieved by integrating a few off-the-shelf components such as a Raspberry Pi 3 model b+, a camera and LED lights into the concept. The concept was mainly developed by the members of the 2021 DeTectUs team, inspired by the most current literature. We benefited from the knowledge and experience of Susan Ibi Preus for the LFA technology and Kaspar Heine Jessen Jürgensen for device prototyping, both from the NaBIS group from DTU Bioengineering.

Supervisors As supervisors of this year's SensUs team from DTU, it has been a pleasure to see the team work towards producing a functioning biosensor for Influenza A. The team has been extremely independent. As supervisors we provided them with the general information at the beginning of the project and suggested a few options that they could look at. From then onwards, the students have made their choices by themselves, with little involvement from us or other members of our group. The only guidance received involved help with ordering and general introduction to lab work. Therefore it is very impressive that they have actually managed to produce a functioning biosensor, incl. a cartridge, a device reader and software, all working together to translate the signal into a concentration. Also considering that there was a period of lockdown at the university, where lab work was limited. We were impressed by how they divided themselves into groups, each working on a specific aspect of the sensor, building on their specific expertise. What the students have achieved is quite close to a final product, by using a combination of off-the-shelf products and home-made sensors. The team has also been in touch with several other people outside our group, and they have already identified a new market for their sensor that they would like to exploit further. They have talked to relevant professionals and were able to confirm that their product would be highly relevant for this market.
All in all we are very impressed with the team and their work this year.

Winnie E. Svendsen, Maria Dimaki, Lorenza Foglia, Mykha Semenov

5 Translational potential

This section addresses the translational potential of the biosensor that has been made by DeTectUs. The first part of the section describes the chosen market opportunity, health monitoring of larger swine populations, while the other parts describes the stakeholder desirability, business feasibility and financial viability of the proposed venture.

5.1 Market description

The business opportunity that the team has decided to pursue is based around Point-of-care (PoC) testing of swine in larger pig farms. This option was chosen based on an interview that was conducted with Rikke Søgaard, a researcher and resident veterinarian at DTU. They are the head of Svin: Objektiv Sundhedsovervågning (SOS), a novel initiative from DTU (Valdimarsson, 2021). This interview revealed the market need for a rapid PoC test that can be used at pig farms to prevent outbreaks.

When a litter of piglets is born, they remain with their mother for approximately 4-6 weeks. Once a weight of approximately 5 kg is achieved, the litter is moved into a bigger stable with several other litters. The pigs will stay here until they mature and are later moved to an even bigger stable for adult pigs. When this happens, the individual pig is typically separated from most of their litter. This means, that the pigs in farms intermingle with one another, which creates the perfect staging grounds for an outbreak without any testing.

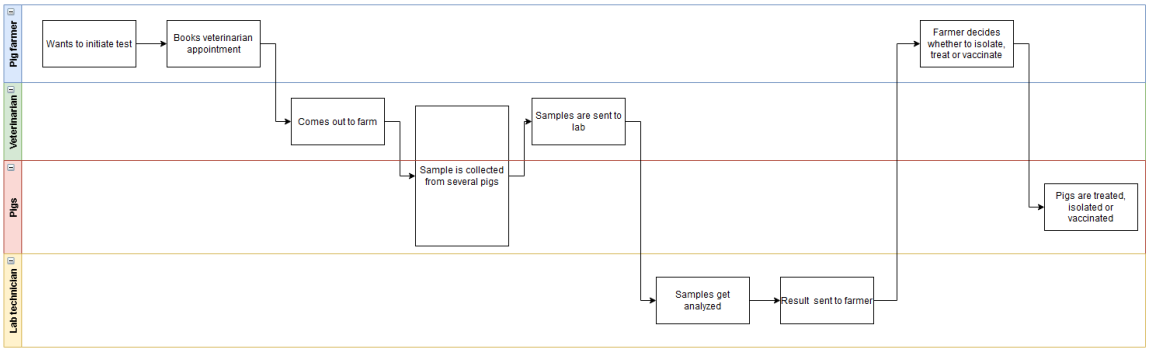


Figure 6: User journey for regular testing of a swine herd

As it is right now, most of the pig herds in Denmark only get tested when the farmer suspects that their pigs might be infected with the influenza virus, or another disease which they can treat with antibiotics. This process can take up to a week from start to finish as shown in 6.

Outside of the inherent risk of a highly contagious sub-type of influenza developing, the result of pandemic outbreaks of influenza in pig farms also pose the threat

of severe economic damage. While the pigs rarely die from being infected, they grow considerably slower than if they were healthy - decreasing the farmers revenue. Infected sows are also at a higher risk of having spontaneous abortions and stillborn piglets, which decreases the cost-efficiency of the sow. In addition to this, the farmer also has to pay for additional veterinarian visits for the sick pigs, as well as having to reserve sections of the farm in order to be able to isolate infected pigs.

5.2 Business model canvas

During the past months, DeTectUs and SOS have been discussing a merger of the two, in order to offer a full "package" to swine farmers including both rapid tests for diagnostics and elaborate data for production optimization.

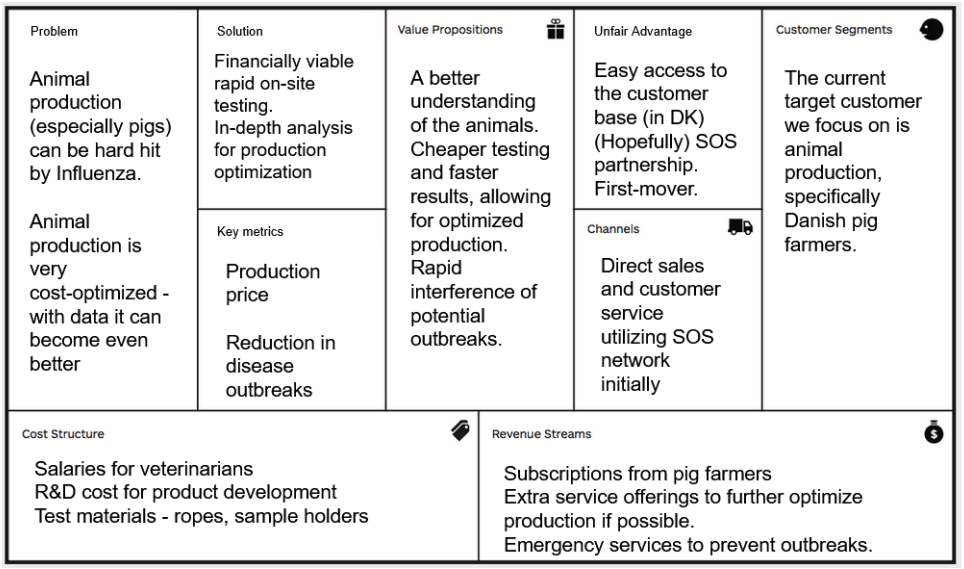


Figure 7: Business Model Canvas

The business model of DeTect-SOS is to develop test kits and services for both diagnostics and surveillance purposes of swine herds. The tests and analysis of them are sold to swine farmers, who in most cases will be able to perform the tests themselves. In some cases an invasive test is needed for diagnostic purposes, in which case a veterinarian will visit the farm to conduct the test. The combination of these tests result in higher quantity and quality of the herd's health, will result in less disease outbreak and optimized production.

5.3 Stakeholder desirability

The first crucial stakeholder is SOS, as we wish to partner with them. They have been very supportive, and helped us a lot in the project overall. For SOS it is naturally important that the team can deliver the promised biosensors.

The second stakeholder are the farmers themselves. Swine farmers wish to optimize herd health and thereby production and profitability, which is the obvious incentive to use DeTect-SOS' service. The results of SOS so far is a good indicator that swine farmers find the service useful and worthwhile. Furthermore, there are a lot of regulations a swine farmer has to abide by, including regular visits from a veterinarian, on average once per month, to check up on the herd's health. Sometimes the diagnostics done by the veterinarian are fairly simple, and with DeTect-SOS, the swine farmer should be able to do some tests themselves, as long as it is non-invasive.

5.4 Business feasibility

This business idea and project have a lot of hurdles yet to jump before becoming reality, especially as it is to some degree detached from the original scope of detection of influenza in human saliva. The first step is to continue product development. To do the product development, some funds will be needed, so more funding is also needed. Before initiation of the product development though, a more thorough customer development process would be beneficial. This should focus on what the swine farmers and veterinarians deem the most needed rapid test, which should also be the most profitable. Once this has been established, the technical team can continue product development. However, initial research for product development can easily be initiated in parallel with the customer development.

5.5 Financial viability

In order to judge the financial viability of the venture, the market size must first be determined, and we have decided to measure this in number of swine and swine farmers. In EU and UK there are 256m swine (total available market). Denmark has 18,1m swine split among 2970 farmers (serviceable available market). The aim is to service 1/4 of the danish market, meaning 740 farmers (serviceable obtainable market). By assuming that four of the team members proceed with the development of the venture in the near future, a budget was made, which includes an overview of the costs, estimated subscriptions and break-even calculations for the first 6 years of operation. The calculation assumes a yearly subscription rate of 24.000 DKK, for approximate 180 tests yearly and the variable cost for each test being 35 DKK. The venture should be able to break even around year 3 with a total needed investment of 2.5 million DKK. At this point the venture would have a yearly turnover of 2.8 M DKK. Should the team be successful in obtaining the goal of servicing 1/4 of the Danish market, it would result in 17.8m DKK revenue/year.

6 Team and support

6.1 Contributions of the Team Members

Mykhaylo has been one of the two team captains and has been focusing in the biology part of the project as well as helping out with the design of the LFA design. Lorenza has been one of the two team captains and has been focusing on research and development and managing the supplies for the project, making sure we did not run out of anything! Arshwinth has worked on the LFA assay development, mainly on the planning of experiments and the assessment of the technical feasibility. He also performed initial training on procedures and equipment while providing consultation regarding any doubts. Rikke has worked on the development of the LFA assay, together with research of the cartridge design. Dayana helped with the detection and prediction algorithm as well as help with LFA assays and literature revision for the design of the LFA. Katarzyna worked on the LFA sensor development, as well as on drop deposition techniques for the LFA assay. Paolo has worked on the code to detect the colour change in our assay and has been providing support in the biosensor development. Mads has been working on prototyping and device development mainly on Fusion360 and has been troubleshooting all the problems that rise up with the device. Siddantha Mohammad has been part of the device team working on setting up the raspberry Pi and has developed an interface to display data on our client's phones. Frederik has been working mainly on the translation potential. This have involved researching various business opportunities, and conducting interviews with relevant stakeholders. William has been working on finding the best possible real-life application for our teams biosensor. This coincides with defining the translation potential of the team. Together with Frederik they have been responsible for securing funding to secure materials for product development and covering travel costs.

6.2 People who have given support

Special mention to Kaspar Heine Jessen Jürgensen and Susan Ibi Preus who have been giving us incredible advice throughout the process and to Christian Vinther Bertelsen for giving us a helping hand during the holiday period when needed it.

6.3 Sponsors

Nano Bio Integrated Systems group (NaBIS): their expertise on nanosensors applied to different applications in the environmental and medical field has been very useful and appreciated over the course of the whole competition. We also received funding from Knud Højgaards Fond and DTU Blue DOT which helped with the buying of materials to perform our experiments.

7 Final remarks

Disclaimer: During the project large delays in the supply chain that were experienced (due to Covid-19 outbreaks). This resulted in inadequate amount of calibration tests needed to evaluate the colorimetric detection / algorithm's accuracy, several tests have been made and more are planned to do this evaluation.

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