Influwegians

University *of* Glasgow

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1. Summary

We primarily sought to create a biosensor to help reduce influenza cases in care home facilities, where flu is a major problem. It kills 22,000 people every year in the UK alone. Care homes are also known focal points of infectivity for the general public as observed during the COVID pandemic. The biosensor cartridges will enable testing of care home staff and residents when they show symptoms, or regularly during the flu season. Diagnosis of positive cases will enable rapid curtailing of the transmission cycle. We believe that our technology will enable a suite of devices to also detect other illnesses in the future, sustainably, for the general population (ultimately as a consumer product).

The following fact is often overlooked given the importance of diagnostics for public health, but in the UK alone, one million COVID tests are sent to landfill every day. The same practice is carried out for a wide range of diseases, amounting to significant waste.

To reduce the impact of diagnostics on carbon footprints, we have created a new biosensor that uses an aptamer-based impedance technique, providing high sensitivity to detect all infected people, and using a simple recycling process allowing the cartridge to be reusable.

2. Biosensor system and assay

2.1 Molecular Recognition and assay reagents

Although whether the binding performance (affinity & specificity) of aptamers is better than antibodies is a matter of debate, the use of aptamers in point-of-care diagnostics offers significant advantages over antibodies including smaller molecule size, rapid and scalable *in vitro* manufacturability, lyophilization for long-term storage and transport at room temperature, as well as ease of controlled chemical modification. The V46 DNA aptamer developed by Bhardwaj et al[1] forms the molecular recognition element of our biosensor. Selected for its high affinity (K_D of 19.2 nM) towards both the Hemagglutinin (HA) stem region as well as the whole H1N1 virus, V46 is suitable for our high-sensitivity impedance spectroscopy (EIS) assay[1].

We use aminopropyl silane covalent attachment to functionalise our substrate with the V46 aptamer. Addition of an ethylene glycol increases the hydrophilicity of the overall chain, which has been proven to improve binding affinity of aptamers[2]. Both the use of hexamethylene and the thymidine spacer have been shown to enhance the accessibility of an aptamer to its target by increasing the distance between the surface and the aptamer's binding site[2][3].

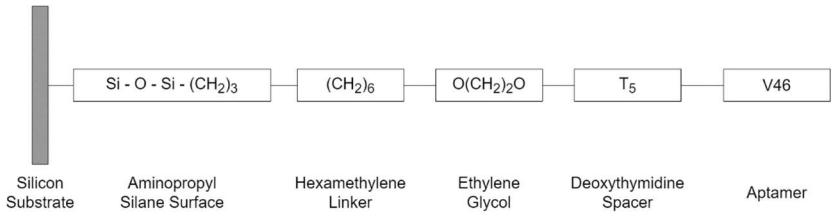


Fig. 1: The main components of the surface functionalisation design

2.2 Physical Transduction

The impedance change of the aptamer-based biosensor can be evaluated by measuring the current response upon the application of a small AC voltage (10mV ~ 100mV). According to the Gouy-Chapman-Stern model, a double layer forms on the metal/electrolyte interface of the working electrode when applying the alternating voltage[4]. The double layer consists of Helmontz layer and Gouy-Chapman layer, where the former is a compact layer of immobile ions strongly attracted to the electrode surface while the latter is a diffusive layer of mobile ions near the electrode surface[5]. Our design is a non-Faradaic impedance biosensor without redox coupling probe, in which the impedance change mainly consists of the change of capacitive component.

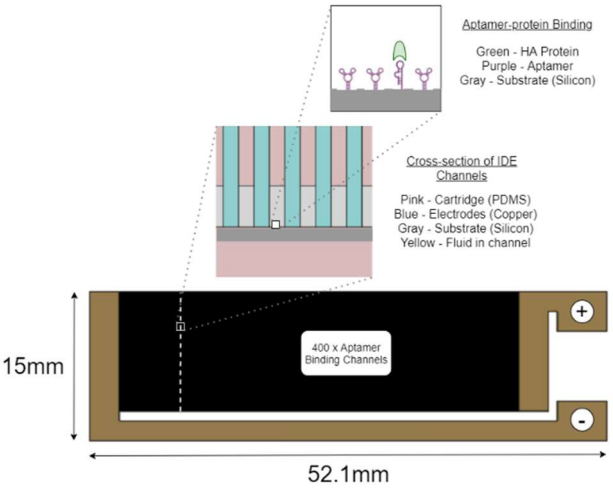


Fig. 2 (Top) conformational change due to aptamer-HA binding (Middle) cross-section view of IDE channels. (Bottom) dimensions of the whole IDE electrodes.

The aptamers are immobilized between two adjacent IDE fingers, see Figure 2. When the HA protein binds to the aptamer, the formation of the complex changes the resistance and capacitance of the circuit, primarily due to the addition of charges from the HA protein, but also the steric impact of the binding (the protein prevents free change movement from the buffer). Our model also suggests that there is a component arising from the conformational change of the aptamer upon binding, but this has not been studied in detail in literature and should be validated by lab experiments.

2.3 Cartridge Technology

Our cartridge consists of two laminated PDMS layers and a thick dry adhesive layer, and an IDE made of gold with copper connections. The sample input chamber (Fig. 3a) is designed to be smaller than the sample volume of 100 µL, so that when the sample is pipetted in, a droplet forms above the chamber. The surface tension created as a result helps to further drive the flow that is already in motion due to capillary action[6].

The eight buffer reservoirs in the cartridge each hold one single-use PBS buffer pouch (Fig. 3b) After the sample has been driven through the entire cartridge and has emptied into the drain tanks on either sides of the IDE, one reservoir is

pushed, releasing PBS to wash any remnants of the saliva sample out of the IDE channels. This allows the impedance reading to be taken without interference from other macromolecules in saliva. Each reservoir holds around 50 µL of PBS, which is sufficient to wash the entire cartridge. Once pressed, this reservoir is now used and its seal will stay depressed, indicating the number of times the cartridge has been used. Eight buffer reservoirs allow for eight uses of each cartridge. After each use, the cartridge will undergo heat treatment and subsequently chemical treatment to enable its reuse.

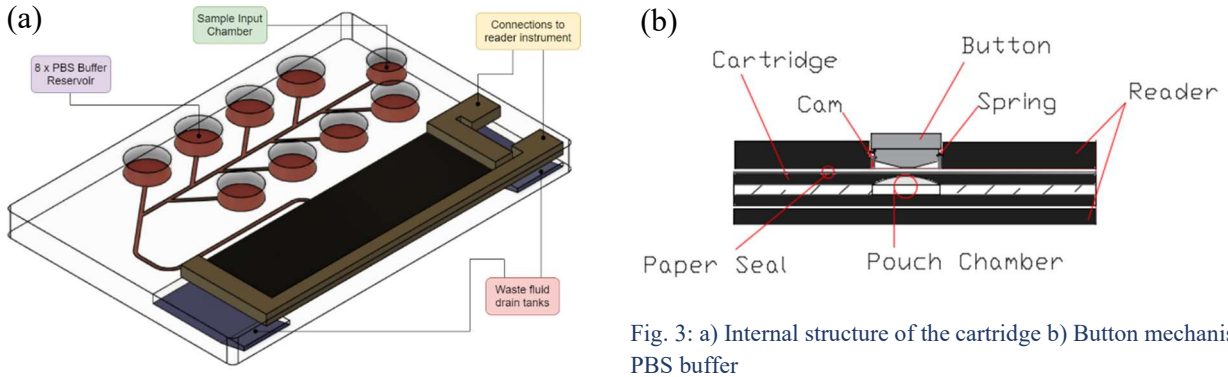


Fig. 3: a) Internal structure of the cartridge b) Button mechanism for the release of PBS buffer

2.4 Reader Instrument

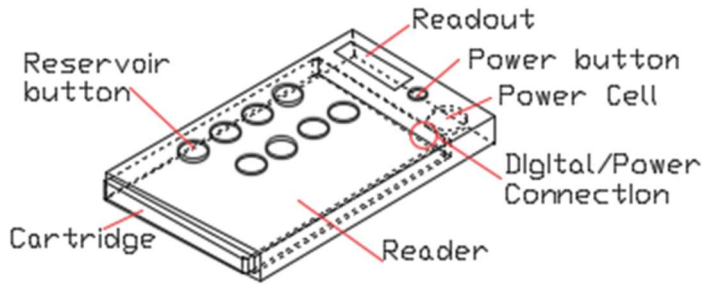


Figure 4: The reader unit with a cartridge inserted

The cartridge is powered by insertion into the reader via the digital/power connection to the electrodes. The reader unit contains the readout screen, power cell, 8 transparent reservoir buttons, and a PCB to handle analysis of the IDE signal.

The buttons on the casing have a sprung 'click' mechanism, similar to a miniaturised version of the mechanism of a ballpoint pen (Fig. 3b). The buttons cannot be permanently depressed, as the cartridge must be ejected for cleaning, so practicality demands a reversible motion such as depressing after the process is finished, so the user can remove the cartridge from the reader. The buttons are transparent, revealing the paper seal of each reservoir above the pouch membrane; once punctured, the pouch membranes are depressed by the button and the torn seal shows the reservoir has been used.

2.5 User Interaction

Our stakeholder engagement highlighted the need to create an app that would be simple and easy to use not only by patients, but also **by healthcare professionals** (including employees of care homes), with minimal training in (molecular) diagnostics. Other constraints highlighted were the need for **privacy**, as the results should not be accessible by third-party organizations, as well as **trust**, meaning that the results should be robust. The Android app provides step-by step test instructions for sample addition and cartridge washing. Firstly, the user dispenses the sample to the inlet. After it flows onto the surface of electrode, the electronic circuits controlled by an Arduino detect the change in impedance. Subsequently, the result is transmitted to the app via Bluetooth and displayed on the mobile phone. Lastly, the user will be instructed with steps to regenerate the cartridge for reuse.

Fig. 5: Home menu of the app, showcasing the four main sections

The app also enables to record relevant personal information, technical contact, and frequently asked questions (FAQs) with answers, are also available. A straightforward menu was chosen as first screen in the app as can be seen in Figure 5. Its simplicity and ease of use was motivated by the need to work time efficient of the target audience, nurses.

3. Technological Feasibility

3.1 Binding Surface Regeneration Cycle

Regeneration of our HA binding surface involves 3 main steps. Firstly, the aptamers must be reversible denatured using heat to detach the bound HA from their aptamer complexes. This is achieved using a heat treatment process of 95°C for 10 min. 95°C is typically used in most reusable aptamer-based biosensors in research, however we propose using boiling water in an insulated container to achieve this safely in a non-clinical setting. Next, 7M Urea is added to the input chamber to wash all of the channels, removing the unbound HA. As a chaotropic agent, it also disrupts and washes away any non-covalently bonded macromolecules along the sample delivery channels, ensuring that these will not affect future tests; this has been used effectively for reusable biosensor concepts in the past [7]. Finally, the cartridges are left to dry and the aptamers to renature at room temperature for 15 min. This allows the cartridge to be reused or stored for later use. Overall, we estimate the full regeneration process to take about 30 minutes.

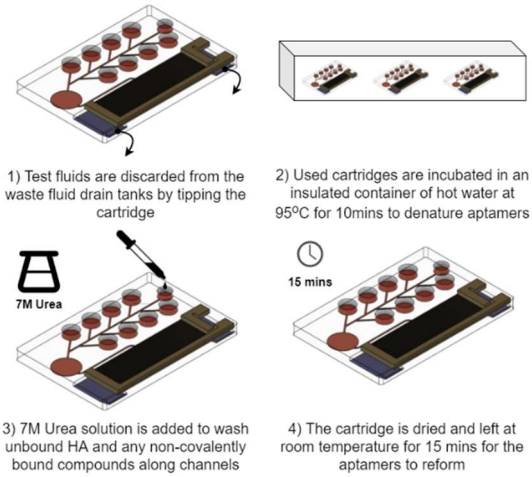


Figure 6: Steps involved in the cartridge regeneration process

3.2 Optimizations of aptamer binding and surface density

Another advantage of using aptamers is that more of them will fit over a given electrode surface area compared to antibodies. Typically, antibodies are about 5 times as large as aptamers, so we can fit 5 aptamers in the area that one antibody would occupy. This combined with controlling the linker molecule length allows us to have a higher density of binding sites for HA on the electrode without the need for expensive surface topographies or nanoparticles. We chose to use 5 thymidine units (T_5) for our spacer length as this has been shown to provide the optimum binding efficacy without compromising surface density of aptamers[3].

3.3 Resistance to Nuclease Degradation

A common problem associated with the use of aptamers in diagnostic applications with body fluids is their vulnerability to attack by nucleases. Exonucleases I and III are the most common in human saliva; exonuclease I poses the greatest threat to aptamers due to its high affinity for single-stranded DNA (ssDNA) molecules.

Methods to reduce nuclease degradation of aptamers include the use of nuclease inhibitors, structural changes, ligation or addition of terminal functional groups[8]. In our biosensor, we use aptamers made with modified L-DNA nucleotides. Known as 'mirror aptamers', they are produced from the synthetic L-enantiomer variants of the naturally occurring D-nucleotides, which makes them highly resistant to natural nucleases which target D-nucleotides. Being mirror images, L-aptamers display nearly identical physical, chemical and structural properties to their D-aptamer counterparts; thus, they provide ultimate protection against nucleases without compromising the aptamer's sensitivity and specificity. Figure 4 shows the results of a nuclease resistance test with exonuclease I and III at 37°C for 45 minutes[9].

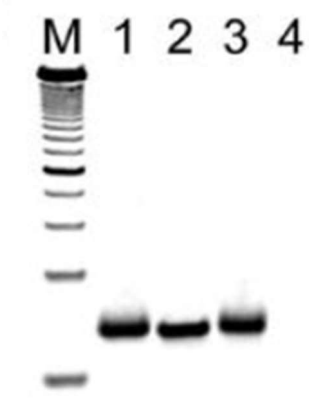


Fig. 7: Nuclease resistance test of a J1four-arm junction on gel electrophoresis; Lane M- 25bp DNA Ladder; Lane 1 & 3 – L-DNA J1 before and after exonuclease treatment; Lane 2 & 4 – D-DNA J1 before and after exonuclease treatment

Using the approximation that each test will involve less than 5 minutes of IDE exposure to the saliva sample (after which the heat treatment will denature any nucleases remaining in the channel residues), we estimate that our immobilized aptamers will be able to withstand at least 9 tests before any notable loss of performance occurs. Practical experiments are needed to characterise the degree of aptamer deterioration and determine the feasibility of their use beyond 9 tests.

3.4 EIS Modelling

One complex between aptamer and HA protein is equivalently modelled as a parallel RC-circuit. Within one unit cell of IDE electrodes, there would be N*M complex units. The change of complex unit accumulates together to contribute the impedance change of one unit cell, and all the unit cells are in parallel to make up the entire system. The biosensor can be modelled through the equivalent circuit shown in Fig. 8, where C11 and R11 represents the resistance and capacitance respectively in one complex.

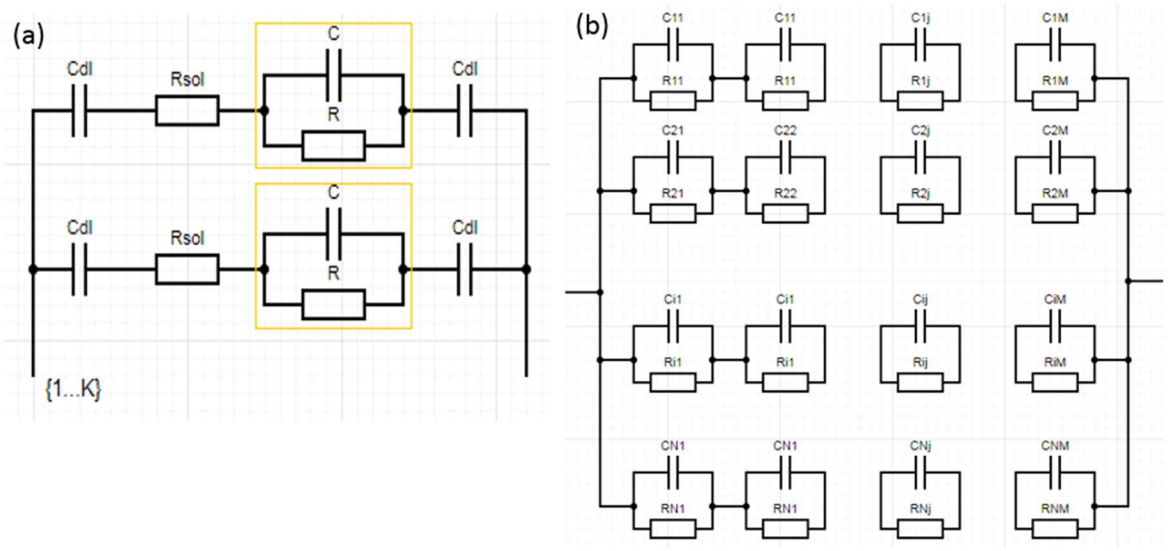
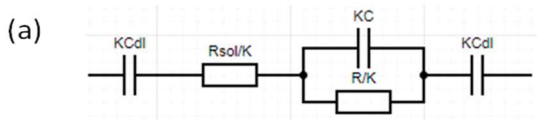


Fig. 8 (a) The equivalent circuit for our IDE biosensor. Cdl: the double layer capacitance, Rsol: the solution resistance, R: the resistance of aptamer complex between aptamer and protein, C: the capacitance of the aptamer complex. K: the number of unit cells of two adjacent IDE electrodes. (b) The yellow highlighted component of (a) detailed as NxM basic units of the aptamer complex.

The impedance change of the entire system can be detected before and after the binding occurs at a specific frequency, where the difference between the two impedance values is maximum in Bode plot of the modelling. The performance of the model was analysed in Python, for which the modelling code is available in Appendix 1. Using a Bode plot (Fig. 9), which puts impedance magnitude versus frequency, the impedance change is derived by simplifying the model considering there is no difference in how the unit cells behave.

Fig. 9(b) demonstrates the frequency which can be used by the stimulating voltage in the electronic circuits of impedance measurement is about 40 kHz, where the impedance difference before and after the binding is maximum.



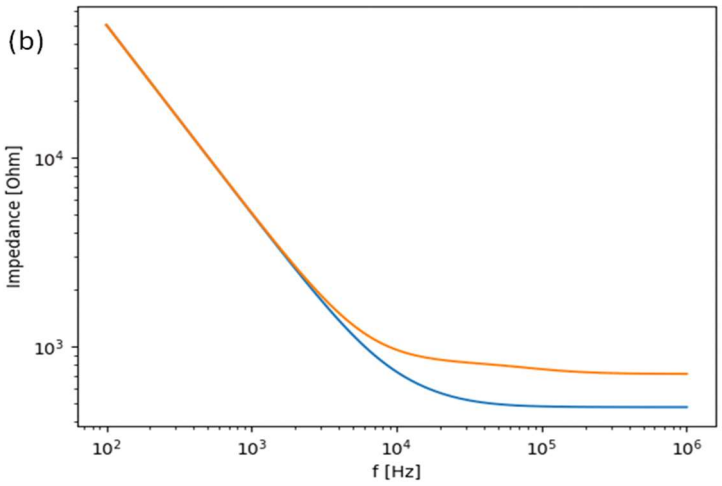


Fig. 9 (a) The equivalent circuit of the circuit shown in Fig. 8(a). K is the number of unit cells in IDE electrode. (b) Bode plot (impedance vs frequency) of the entire system. Component values are assumed from Jacob Lum et al [10]: before the binding (blue), Rsol: 10kΩ, Cdl: 3nF, R: 1kΩ, C: 10nF; after the binding (orange), Rsol: 15kΩ, Cdl: 3nF, R: 3kΩ, C: 1nF.

Assuming that the resistance increases by 50%, the capacitance decreases by 50% and resistance of solution increases proportionally with the number of complex (f: 40 kHz) after the binding occurs, we get a directly proportional relationship between the impedance change and the number of aptamer-protein complexes, represented by one parallel RC-circuit in the circuit matrix (Appendix 2). We have not found sufficient evidence to support such predictions, which could be further identified in the experiments.

4. Originality

4.1 Note from Influwegians

Challenge. The primary constraint of any biosensor is its practical applicability, which translates into performance (specifically, sensitivity). This statement has been repeated to us throughout our stakeholder engagement, stating that sustainability was not a main driver. We strongly believe that although there cannot be any compromise on performance, dismissing sustainability to the background is a missed opportunity. We already see research laboratories 'going green'[11][12] and our NHS initiating new (process-driven) activities to address carbon issues[13]. Consequently, we decided at the outset of the project (after our first initial brainstorming) to focus on potential techniques that could bring in sustainable solutions.

Novelty. Although reusability has been researched and is present in scientific literature, the characterisation is patchy, and solutions are disparate and complex to implement in real-life. We have identified the specific capabilities of aptamers as unique in this context, enabling the surface chemistry to be refreshed easily, but also to create a platform technology that could be expanded for other markers (by changing or adding – multiplexing – other aptamers as they become available). Our unique approach lies not only with considering the capture molecule performance, but also the practical implementation in large scales, which we believe has not been carried out so far.

Summary of the approach. Aptamers have been utilized in reusable biosensors for detecting proteins in past research[14]. However, adapting the aptamer denaturing process to be used in a non-laboratory setting without the need for expert oversight or hazardous chemicals is a unique idea conceived by our team; using heat and washing with a simple buffer, to be then reused. Our approach transcends the current paradigm where healthcare devices are simply used and discarded, which is estimated to have generated 1.6 million tonnes of plastic waste globally per day since the COVID-19 outbreak[15]. As this year commences the COP26 in Glasgow, we were keen to develop ideas that could change the way we think of sustainability in healthcare. We cannot simply ignore the effects of healthcare waste; we must develop and find ideas to tackle it.

Rebecca Bean
Team Captain

Hasitha Senevirathne
Team Captain

4.2 Note from Supervisor (Julien Reboud)

The team has been very keen, from the outset, to incorporate sustainability aspects in their designs. I believe this is due to the Team Captains' leadership and the general discourse currently in the University of Glasgow, linked to the COP26 taking place in the city in a few months' time. This approach has provided the team with a significant challenge, especially due to the fact that many in the community do not believe that the impact of diagnostic testing on the carbon footprint is significant. As discussed in this report, this was the position of many of the stakeholders.

Although I have been aware of research in the area of reusability, mostly through surface chemistry (for example with detachable linkers), or through using specific washing buffers to ensure that no target is left on the surface after use, the team did their own research and that drove them to the use of aptamers (which we have not yet used in our lab) and washing/recycling, **completely independently**. The rest of the approach (fluidics, electrical detection method), although coming from robust engineering design thinking, is conventional. But here again, I must stress that the Team was not directly supported by our researchers. Although we have extensive experience in the area, there is no active on-going research at the moment in our group on these techniques. All in all, I am very proud of how the team focussed on a difficult challenge and provides a workable solution.

Julien Reboud
Supervisor

5. Translational Potential

5.1 Business Model Canvas

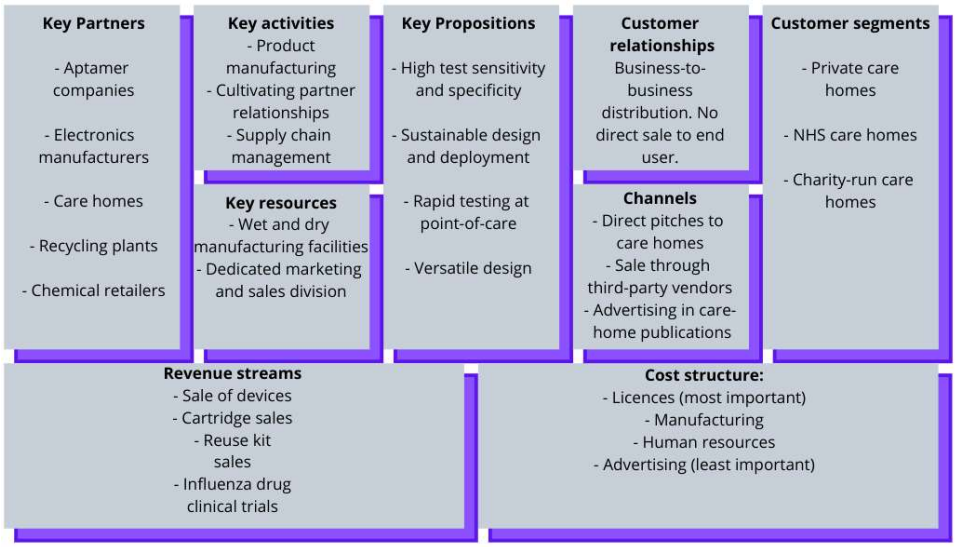


Fig. 10: At the centre of our Business Model Canvas sits an overarching summary of our key propositions. On the left are the partners, resources, and activities which will enable us to fulfil these propositions. On the right lies a description of our channels to, and relationships with, our end-users. Finally, at the base sit the cost structure and revenue streams which provide insight into our financial priorities (more detail provided in Section 5.5)

5.2 Market Description

Initially, care homes will be targeted as the primary customer segment of interest. Care-home residents have a higher risk of mortality from respiratory viruses such as Influenza, as seen in Appendix 4[16]. This is primarily due to the advanced age of the residents and high incidence of comorbidities; the high degree of person-to-person contact in care homes could be a further contributor[17]. The COVID-19 pandemic provides an apt analogy for an Influenza outbreak by illustrating the devastating effects that a virus can have on care homes[17]

The provision of healthcare services in care homes is split between care providers who have their own procurement services like Barchester Healthcare, and smaller care homes who work with the local general practitioners. Currently, there is very little in-house testing, with resident samples having to be sent away to labs and test results having to be sent back. Our discussions with both General Practitioners (GP) and care homes highlighted that this limited provision of in-house testing is a technological and processing issue (no test currently commercially available and limited expertise at the home for more complex approaches).

In the UK there are 17,600 care homes from 5,500 care providers[18], which will provide a strong initial market for our biosensor. Once the business has established itself in this market segment, we will expand by directly retailing to the NHS, supplying general practices and hospitals of which there are 9,000 and 1,200 respectively[19][20].

5.3 Stakeholder Desirability

The primary stakeholders benefitting from the introduction of an in-house rapid-testing system are the care-home residents and staff (including management). Besides these, general practitioners will benefit since they would no longer need to expend time and manpower redirecting Influenza tests to specialised labs. Care-home visitors (such as family members) would also benefit since on-site rapid testing would lift the psychological burden of knowing that they could be endangering the residents. Finally, institutions such as insurance companies and hospitals would benefit from a decreased patient load during flu season, since outbreaks will be more effectively managed.

The current diagnostic procedures applied in care homes have been shown to be ill-equipped to manage a pandemic (following the example of COVID-19). From the three interviews we have conducted with care-home managers, staff are suspected to be the primary means through which infections are introduced into the environment. There is no established testing policy for care-home workers. Whilst residents are the only members of the care-home community to receive diagnostic tests for Influenza: this is done as a means of confirming a preliminary diagnosis by a medical

professional, so is done after the onset of symptoms. As such, in the context of an outbreak, the delay between infection and diagnosis would likely be too large to prevent transmission. Indeed, care homes tend to implement more stringent regulations upon confirmation of two individual cases.

The delay between infection and diagnosis is further prolonged by the cumbersome logistics underpinning the process: after a care-home nurse has collected the requisite swabs, they must be sent to a GP before being sent onto a virology lab for analysis. This increased delay again (sometimes lasting days) enhances the potential for transmission, thus making containment of outbreaks more challenging. Thus, a device that enables rapid, on-site testing will significantly benefit both the primary and secondary stakeholders. Seasonal outbreaks would be more easily contained, and patients would receive treatment faster thanks to the rapid diagnostic capabilities of our device. Furthermore, the introduction of in-house testing would alleviate the administrative burden placed on GP surgeries, since they would no longer need to forward tests to specialised laboratories.

Naturally, high-sensitivity and specificity must precede a fast diagnosis. Correspondingly, our biosensor will provide the same analytical and clinical performance as laboratory-based test, which report sensitivities ranging from 92%-99% and specificities ranging from 97%-100%[21].

A final integral element of our value proposition is our pledge to promote sustainability at each phase of the product life cycle. The need for such a commitment is evident in the enormous amount of plastic waste that has been generated by rapid-testing kits for COVID-19. Indeed, the obtention and processing of a single patient sample generates over 37g of plastic residues, the majority of which (~97%) will be incinerated[22]. When considered alongside the vast quantities of tests currently being demanded, this amounts to a tremendous environmental burden since plastic incineration is known to contribute significantly to atmospheric pollution.

Our biosensor will counteract this by combining an innovative, eco-friendly design with a strategy for deployment and expansion. The device will feature a cartridge-based design which employs recyclable materials wherever possible. This will be complemented by using washing kits, allowing the cartridges to be reused on site. By way of these innovations, we will help care-homes adapt into eco-friendly organisations that are able to thrive in the twenty-first century. Furthermore, when we later expand into the NHS, our environmentally friendly initiatives will be well-aligned with the NHS's aspiration to reach net-zero emissions[23].

5.4 Business Feasibility

We will implement the strategy through partnerships with a recycling plant, enabling us to benefit from high volume processing and existing supply chain and logistics benefits. However, this is only economically feasible at scale. Therefore, initially in-situ pilot washing would be implemented. The process consists of a water-based heat treatment (95°C) followed by a chemical wash. We will partner with our customers to explore either a service model (with our technician going onsite for processing) or more extensive training of specific care home personnel. Each washing package comes with the reagent for rapid recycling (dry urea) and the insulated container for the heat treatment.

The manufacture of the device relies on the delivery of three different sections: (1) aptamers and chemical treatment, (2) electronics and user interphase components and (3) the physical body of the device and cartridge printing. Although the product could eventually be deployed internationally, the initial market scope remains local, meaning that all the manufacturing sites are expected to be located within the UK. We have selected our partners and suppliers by considering which supplier would provide us the best economic returns, whilst prioritising our vision and team values.

SLC (Scientific Laboratory Suppliers) is the largest independent supplier of laboratory equipment in the UK. Its commitment to sustainability along with its large professional network and competitive fees makes it an attractive supplier. Considering that the product would be initially launched into a specialised market, the injection moulding of the cartridges and external cases has been decided to be done through a third party as this results in the most economically viable option. Polymermedics, which empowers the choice for sustainability, is our first option supplier.

5.5 Financial Viability

As stated above, the manufacturing would be outsourced to Polymermedics due to its economic viability, giving a cost per unit at £2.00. Each cartridge will be priced £40.00/unit with a reuse kit with 8 pipettes and urea sachets for sample loading and cartridge washing. The reuse kits allow for each cartridge to be used 8 times which sets a price of £5.00 per test for the customer. With the reuse kits billed at £1.00/unit, the profit margin for each cartridge will be £30.00 and this will be our primary revenue stream. For the sensor itself, the manufacturing will cost £40.00/unit giving a profit margin of £60.00.

In the current biosensor market, competitors are priced at an average of £14.50 per test[24] whereas ours is £5.00, providing a very competitive price for flu sensors market (excluding the price of the sensor itself which is a £100.00 one-off). The product will be sold directly to care providers such as Barchester Healthcare and HC-One Ltd, via the company sales team.

Since we will be selling to care providers and the NHS, FDA approval is required by Public Health England[25] for POTC's. This is a significant investment, since the average cost of FDA Class II medical device being £22.6 million[26]. Nonetheless, this initial investment is a prerequisite to entry to the healthcare market.

As seen in Appendix 5 the company will break even in the second year of operation, this can be noted by the crossover point. The financial plan in Appendix 3 includes the initial customer segment of care homes, and then expands to the NHS, supplying GPs and Hospitals after the second fiscal year. The estimations on sales were carried out by looking at the market size and determining how many tests are regularly carried out, as well as the numbers of staff in the care homes. NHS sales were estimated by analysing current GP ILI (Influenza-Like Illness) consultation data[16].

6. Team and support

6.1 Contributions of team members

Bei Zhan: Entrepreneurship team
Finlay McAndrew: Team Promotional Person, business plan development, app development and presentations
Gabriel Dzharadat: Commercialization, event planning and pitching
Hasitha Senevirathne: Team captain, aptamer selection, sustainable development, molecular assay and CAD design
Ilse Jansen: Microfluidics design, user interface app development, SensUs event and presentations
Jacob Skipper: Microfluidics design, sustainability, user interface app development, sketches and diagrams
Jinfeng Liu: IDE electrode and circuit design, impedance modelling
Lucía Muñoz Bohollo: User interface app development
Matteo Rochon Cocchiara: Business plan development, SensUs event
Rebecca Bean: Team captain, administration, sustainable development, CAD design, animations and presentations
Sofia Herrero Barros: Business plan development, aptamer selection

6.2 People who have given support

Special thanks go out to our supervisor Julien for his extensive help throughout the competition and in inspiring us during this year of online meetings. We would also like to thank all the Alumni and partners for their support and guidance with the SensUs competition;
Abi Graham and Wenshu Xu (TTP) with whom the team has had partner discussions about the initial design and viability.
Willem van Velzen (PalmSense) for the discussions about electronic development, reusability and use of Arduinos.
Ernst Lindhout (Future Diagnostics) for discussions about the initial design.
Robert Jan and Toon Stilma (Roland Berger) for consultancy on the business plan and development of start-up companies.
Menghan Zhan (PhD student at the University of Glasgow) for key discussions about the potential of aptamers, their structure and chemistry.
Marycarmen Flores (SensUs alumni, SenseGlasgow 2020) for her experience with SensUs, advice on the business plan, market research and how to improve the team organisation.
Mr Bernard Hoey (Technician in School of Engineering) for soldering our RS components circuits despite period of COVID19 restrictions with limited lab access
Marion Anderson and Mel Sherwood for the Pitchtastic Pitching Professional Event.

6.3 Sponsors

We would like to thank RS Components for their funding awarded to the team through the RS Grass Roots Student Project Fund. This award has been helpful in purchasing electrical equipment during the initial development stage of the project.

7. Final Remarks

COVID-19 roller-coaster. As the project started almost a year ago, the future looked uncertain, but we all (including our supervisor) were hopeful to be able to carry out parts of the work in the lab at some point during the year. Unfortunately, this has never been the case, although, as lockdowns came and went and we learnt of other teams being able to access labs, we kept hoping. This has affected the team's morale and the project became quite a roller-coaster ride. However, we are proud of what we have achieved so far without any lab access and believe that the project has great potential.

Future access. We hope that lab access may become possible in the upcoming year to enable our team to test our key concepts. Following the success in pitching competitions last July, the team is keen to further apply to business and pitching competitions. With the support of the SensUs organisation and our university's biosensor society GUBiosense we hope to both further develop our product and, most importantly, increase awareness of sustainability within the healthcare sector.

8. References

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9. Appendix

Appendix 1

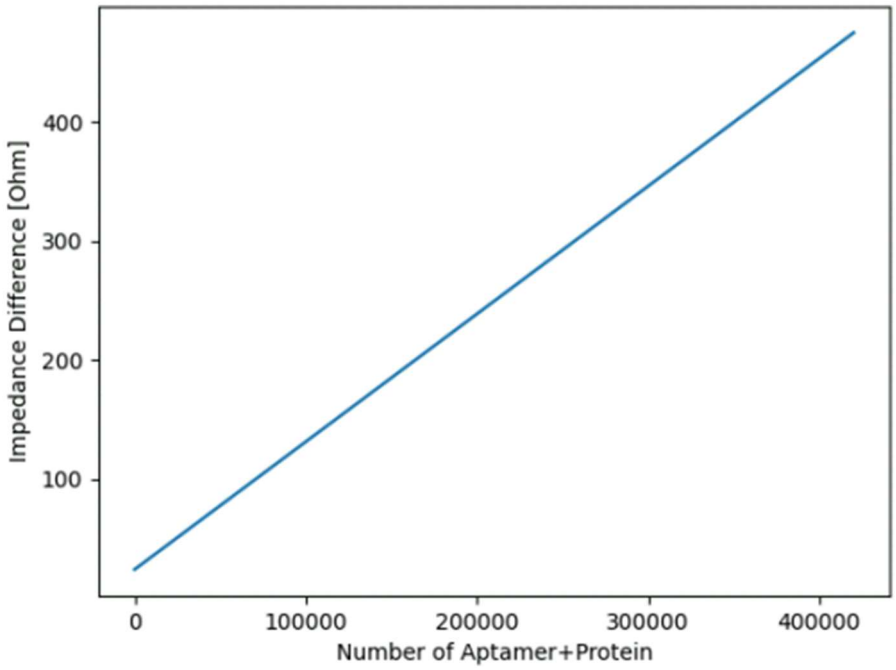
The Python code used for modelling the impedance change.

```
import numpy as np
import matplotlib.pyplot as plt
import cmath
import math

N = 100 #number of frequency point

def Parallel_Conn(Z1, Z2):
    Z = (Z1*Z2)/(Z1+Z2)
    return Z

def Serial_Conn(Z1, Z2):
    Z = (Z1 + Z2)
    return Z

f = np.logspace(1, 6, N) #fequency from 1e1 to 1e6 Hz, N frequency points sampling
w = f * 2 * np.pi

K = 20
Rs = 10000/K # 10k
Cdl = (3e-9)*K

Ra = 1000/K
Ca = (1e-8)*K

Z_Cdl = [complex(1,1)]*N
Z_Ca = [complex(1,1)]*N

for i in range(0, N):
    Z_Cdl[i] = complex(0, -1/(w[i]*Cdl))
    Z_Ca[i] = complex(0, -1/(w[i]*Ca))

################################
# Cdl + Rs + (Ca || Ra) + Cdl
################################

#initialized as complex number
Z1 = [complex(1,1)]*N
Z2 = [complex(1,1)]*N
Z = [complex(1,1)]*N

for i in range(0, N):
    Z1[i] = Serial_Conn(Rs, 2*Z_Cdl[i]) #serial conn
    Z2[i] = Parallel_Conn(Ra, Z_Ca[i])  #parallel conn
    Z[i] = Serial_Conn(Z1[i], Z2[i])    #serial conn

Z_Mag = [1]*N #initialized as real number

#calculate the mag by frequency point
for i in range(0, N):
    Z_Mag[i] = np.sqrt(Z[i].real*Z[i].real + Z[i].imag*Z[i].imag)

#plot the figure
plt.figure(1)
plt.loglog(f, Z_Mag)
plt.xlabel('f [Hz]')
plt.ylabel('Impedance [Ohm]')

Rs = 15000/K # 15k
Cdl = (3e-9)*K
Ra = 3000/K
Ca = (1e-9)*K

for i in range(0, N):
```

```
    Z_Cdl[i] = complex(0, -1/(w[i]*Cdl))
    Z_Ca[i] = complex(0, -1/(w[i]*Ca))

for i in range(0, N):
    Z1[i] = Serial_Conn(Rs, 2*Z_Cdl[i]) #serial conn
    Z2[i] = Parallel_Conn(Ra, Z_Ca[i])  #parallel conn
    Z[i] = Serial_Conn(Z1[i], Z2[i])    #serial conn

Z_Mag = [1]*N #initialized as real number
#calculate the mag by frequency point
for i in range(0, N):
    Z_Mag[i] = np.sqrt(Z[i].real*Z[i].real + Z[i].imag*Z[i].imag)

#plot the figure
plt.loglog(f, Z_Mag)
plt.xlabel('f [Hz]')
plt.ylabel('Impedance [Ohm]')
plt.show()
```

Appendix 2

The relationship between the impedance change and the number of aptamer-protein complex.



Appendix 3

(a) Costs associated with manufacture and staffing throughout year 1

			Year 1			
Revenue stream	price (£)		Quarter 1	Quarter 2	Quarter 3	Quarter 4
Sensor	£100.00		2200	2200	2200	2200
Cartridge	£40.00		52300	52300	52300	52300
Turnover			£2,312,000.00	£2,312,000.00	£2,312,000.00	£2,312,000.00
Costs	cost (£)					
Sensor	£40.00		£88,000.00	£88,000.00	£88,000.00	£88,000.00
Cartridge	£10.00		£523,000.00	£523,000.00	£523,000.00	£523,000.00
Total			£611,000.00	£611,000.00	£611,000.00	£611,000.00
Profit margin			£1,701,000.00	£1,701,000.00	£1,701,000.00	£1,701,000.00

Third party services	cost (£) per Q		Initial Startup costs	cost (£)		Marketing cost
Legal services	£700.00		FDA approval	£22,500,000.00		Advertising
Accounting	£300.00		Nordval approval	£10,000.00		distribution
Consultants/contracto	£1,000.00		CE certification	£25,000.00		total
Machinery servicing	£1,000.00		Machinery + Lab Equipm	£50,000.00		
Total	£3,000.00		Total	£22,585,000.00		
	Cash Flow		-£21,034,300.00	-£19,483,600.00	-£17,809,600.00	-£16,135,600.00

Human resources	cost per month (£)	per Q	Number of staff			
Sales team member	£3,500.00	£10,500.00	4	4	4	4
Researcher	£4,000.00	£12,000.00	4	4	4	4
Marketing team memb	£3,700.00	£11,100.00	3	3	3	3
Quality control	£4,200.00	£12,600.00				
Total			£123,300.00	£123,300.00	£123,300.00	£123,300.00

(b) Costs associated with manufacture and staffing throughout year 2 and 3

Year 2				Year 3			
Quarter 5	Quarter 6	Quarter 7	Quarter 8	Quarter 9	Quarter 10	Quarter 11	Quarter 12
2200	2200	2200	2200	2250	2250	2250	2250
104600	104600	104600	104600	127100	127100	127100	127100
£4,404,000.00	£4,404,000.00	£4,404,000.00	£4,404,000.00	£5,309,000.00	£5,309,000.00	£5,309,000.00	£5,309,000.00
£88,000.00	£88,000.00	£88,000.00	£88,000.00	£90,000.00	£90,000.00	£90,000.00	£90,000.00
£1,046,000.00	£1,046,000.00	£1,046,000.00	£1,046,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00
£1,134,000.00	£1,134,000.00	£1,134,000.00	£1,134,000.00	£1,361,000.00	£1,361,000.00	£1,361,000.00	£1,361,000.00
£3,270,000.00	£3,270,000.00	£3,270,000.00	£3,270,000.00	£3,948,000.00	£3,948,000.00	£3,948,000.00	£3,948,000.00

cost per Q		Infrastructure + op	Cost per Q
£2,000.00		Travel	£1,000.00
£10,000.00		Office expenses	£10,000.00
£12,000.00		ICT	£1,000.00
		total	£12,000.00

-£12,892,600.00	-£9,649,600.00	-£6,406,600.00	-£3,163,600.00	£757,400.00	£4,678,400.00	£8,599,400.00	£12,520,400.00
4	4	4	4	6	6	6	6
4	4	4	4	6	6	6	6
3	3	3	3	4	4	4	4
				1	1	1	1
£123,300.00	£123,300.00	£123,300.00	£123,300.00	£192,000.00	£192,000.00	£192,000.00	£192,000.00

(c) Costs associated with manufacture and staffing throughout year 4 and 5

Year 4				Year 5			
Quarter 13	Quarter 14	Quarter 15	Quarter 16	Quarter 17	Quarter 18	Quarter 19	Quarter 20
0	0	0	0	0	0	0	0
127100	127100	127100	127100	127100	127100	127100	127100
£5,084,000.00	£5,084,000.00	£5,084,000.00	£5,084,000.00	£5,084,000.00	£5,084,000.00	£5,084,000.00	£5,084,000.00
£0.00	£0.00	£0.00	£0.00	£0.00	£0.00	£0.00	£0.00
£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00
£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00	£1,271,000.00
£3,813,000.00	£3,813,000.00	£3,813,000.00	£3,813,000.00	£3,813,000.00	£3,813,000.00	£3,813,000.00	£3,813,000.00

£16,306,400.00	£20,092,400.00	£23,878,400.00	£27,664,400.00	£31,450,400.00	##########	£39,022,400.00	£42,808,400.00
6	6	6	6	6	6	6	6
6	6	6	6	6	6	6	6
4	4	4	4	4	4	4	4
1	1	1	1	1	1	1	1
£192,000.00	£192,000.00	£192,000.00	£192,000.00	£192,000.00	£192,000.00	£192,000.00	£192,000.00

Appendix 4

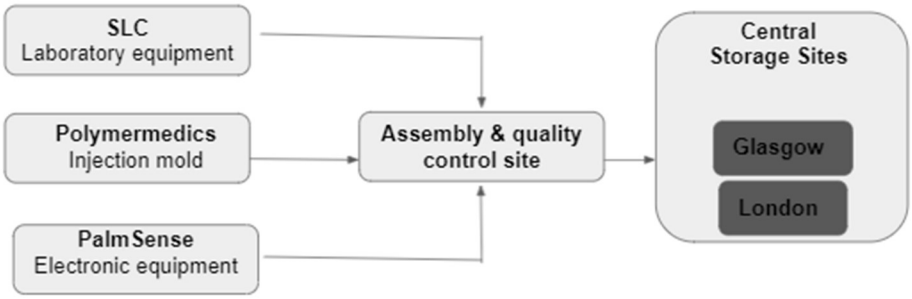
a) Estimated number of excess deaths from Influenza by age group as calculated by Public Health England, 2020 [FM-1]

	7,371	30	8	619	6,048
	15,047	48	15	365	13,480
	22,087	13	4	1,365	19,525
	3,966	63	3	322	2,939
	7,990	55	10	534	6,905

b) Number of outbreaks per institution as recorded by Public Health England, 2020 [FM-1]

	3,936	1,432	2,149	1,114
Institution type				
	2,751	1,013	1,700	875
	257	206	230	162
	656	162	160	61
	126	-	-	-
	146	51	59	16

Appendix 5



Reusability example I: Purchasing only seasonal cartridges over time

The use of a seasonal cartridge implies that once the customer has bought the biosensor, she/he would only need to purchase a new cartridge rather than a full testing package again. Therefore, it increases the reliability of our business as the customer will be most likely to remain using the same product. Similarly, it provides the customer with an economical and highly effective solution as each cartridge will be specifically targeted the seasonal variant.

Appendix 6

Break even chart: The break-even point occurs in the 9th Quarter.