# Imperial College London 2018

# Team Results Document

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**Supervisor and Coach:**Professor Tony Cass, Dr Thao Le

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# Contents

# 1. Team Summary

SensImperial is the Imperial College London team taking part in SensUs 2018, with the ambitious goal of successfully designing a novel biosensor for the last-resort antibiotic vancomycin.

Our team consists of 10 passionate and dedicated undergraduate students, striving to apply their knowledge to solve real-life problems. Our abilities cover a wide range as our team incorporates physical, synthetic and medicinal chemists, as well as biomedical and electrical engineers. This was to ensure that there is a solid understanding of the project benefits in both the biochemical and engineering fields. Also, coming from different backgrounds, we have all learnt from each other and have become more well-rounded researchers, because science and engineering are at their most useful when applied together.

We wanted our sensor to be quick, efficient and easy to use. We employed a colorimetric detection method in a competitive lateral flow assay (LFA). This makes use of the strong antibiotic – antibody binding interaction, which allows the vancomycin molecule to displace a vancomycin-conjugate tagged with a coloured label from the LFA membrane, giving rise to a detectable change in colour intensity. The biosensor then converts this change into the corresponding concentration of antibiotic in blood plasma.



*Figure 1: SensImperial team members*

# 2. Biosensor System and Assay

The purpose of our biosensor is to detect the concentration of the drug vancomycin in patients' blood. This last resort antibiotic shows a high interpatient pharmacokinetic variability; thus, the ideal dose differs for each individual. Our biosensor makes it easy to find and administer the correct dose, which helps prevent overdose and the associated side effects (e.g. kidney failure, hearing loss). By keeping the concentration of vancomycin within the therapeutic range a balance is struck between efficacy and toxicity. Moreover, controlling the levels of vancomycin prevents bacteria from developing antibiotic resistance, a present and concerning threat in the case of last-resort medication.

## 2.1 Lateral Flow Assay Design

Our biosensor uses a lateral flow assay (LFA) to quantitatively detect the levels of vancomycin in a blood plasma sample. A nitrocellulose membrane attached to a plastic backing is printed with two lines, one containing vancomycin antibody and the other containing streptavidin. A bovine serum albumin (BSA) conjugate, containing vancomycin, biotin and gold nanoparticles (AuNPs) was synthesised. The AuNPs are responsible for the conjugate's bright red colour, while the vancomycin and the biotin will bind to the antibody and streptavidin lines respectively. This conjugate and the plasma sample are then washed over the membrane and will travel along through capillary force. Initially, the red conjugate binds to the leftmost (antibody) line. If vancomycin is present in the plasma sample, this will displace the conjugate, which will instead bind to the streptavidin line. As the concentration of vancomycin in the sample increases, the intensity of the red colour on the first strip decreases, and the red colour intensity of the second strip increases. The different colour intensities of the strips can then be correlated to an exact concentration of vancomycin that has displaced the conjugate.
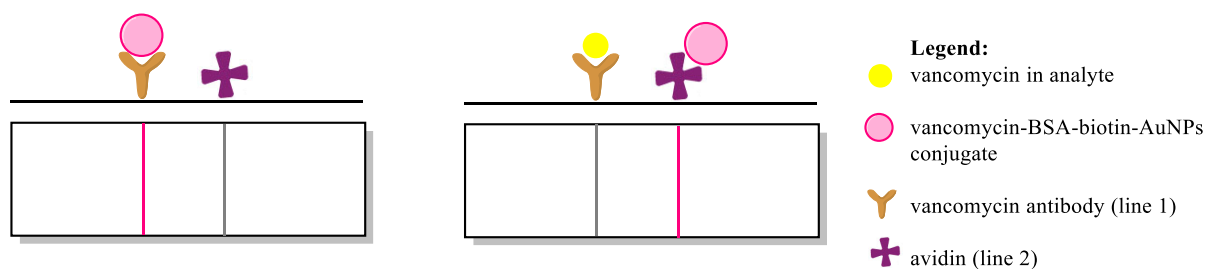


**Legend:**
- vancomycin in analyte
- vancomycin-BSA-biotin-AuNPs conjugate
- vancomycin antibody (line 1)
- avidin (line 2)

*Figure 2: Lateral Flow Assay Explained*


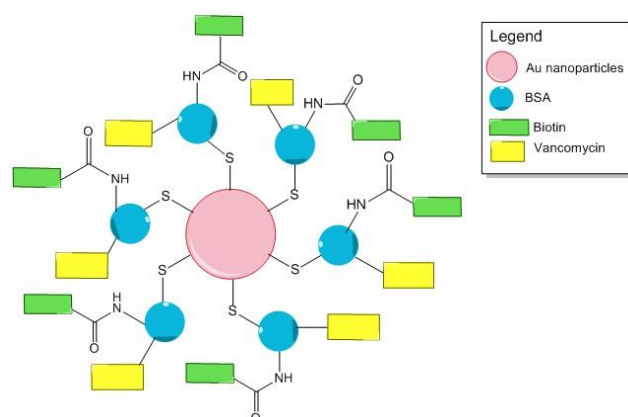
*Figure 3: Complex Conjugate structure*

Legend
- Au nanoparticles
- BSA
- Biotin
- Vancomycin

## 2.2 Design and synthesis of complex

The synthesis of the BSA-biotin-vancomycin-gold conjugates required several steps. Vancomycin was linked to BSA through a standard peptide coupling procedure[1,2] and the subsequent product was purified by dialysis through a 12,000-14,000 MW semi-permeable membrane. The presence of the BSA-vancomycin complex was confirmed using UV-vis spectroscopy, recording the absorbance at 280 nm.

Next, a standard biotinylation procedure[3] was followed to bind the BSA-vancomycin conjugate to NHS-LC-biotin. The product was purified through dialysis and the binding of biotin was confirmed through an HABA-avidin assay.

2-mercaptoethanol was added to the protein conjugate and mixed well before being left to react at 4 °C for 8-12 hours. Excess 2-mercaptoethanol was removed through dialysis. The solution was then added dropwise to 40 nm colloidal gold and the reaction was left to react for 2 days at 4 °C. To extract the gold complex, the solution was centrifuged at 13400 RCF for 1 hour and washed with phosphate buffered saline (PBS). This

procedure was repeated 3 times. The pellet was then dissolvedin the PBS solution and used to soak glass fibre pads to be used in the membranes.

## 2.3 Assembly of membranes

The membranes for the lateral flow assay were assembled by spotting vancomycin antibody and streptavidin onto a nitrocellulose membrane. The membrane was then soaked in a 5% BSA Tween-20 solution in PBS to saturate the unbound positions on the membrane, thereby preventing the conjugate from binding.

The glass conjugate pads were soaked in 5% glycerol solution in PBS for 10 minutes before drying at 37 °C. Once dry, the pads were immersedin conjugate solution and attached to the membrane. The other end of the membrane was fitted with an absorbing wick.

## 2.4 Measuring vancomycin concentrations

To use the membranes, minimal blood plasma solution was pipetted onto the sample pads. The solution travels up the strip by capillary action. The conjugate was washed along the membrane by the blood plasma.

The colour intensity of the membranes was tested using a colorimetric system as part of a camera app using a smartphone. The app images the membrane and measures the intensity of the antibody and streptavidin lines by automatically finding the location of the strips and subtracting the mean value of the measured colour from the mean background colour of the membrane to get a value of intensity that when inserted into the equations of the calibration curves which return a specific concentration for each intensity value. These results can be saved for future viewing in an encrypted file.
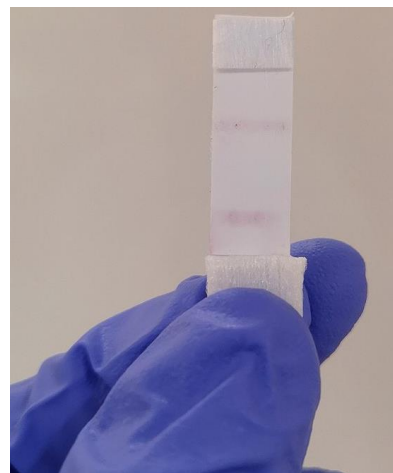


*Figure 4: Membrane showing the 2 coloured test lines*

Multiple vancomycin blood plasma solutions of known concentrations within the required range (5 to 100 mg/ml) were tested to plot the calibration curve (intensity versus concentration). Once obtained, this curve can be used to find unknown vancomycin concentrations from any blood plasma sample using the assembled membranes.

At the time of writing, a neural network is being developed to further improve results. The neural network is being taught with 100 images per concentration that have been taken under a variety of lighting conditions with different smartphones cameras. This will result in a neural network that is specifically trained to tell the difference between vancomycin concentrations in the membrane. The findings from this will supplement the result from the calibration curves to provide a more accurate value for concentration.
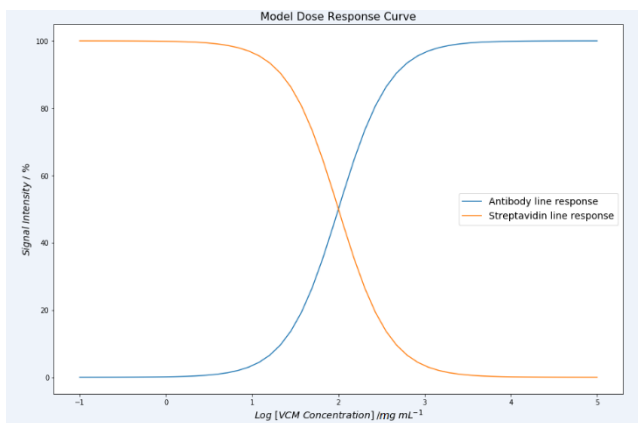


*Figure 5: Smartphone App for membrane analysis - Interface*



*Figure 6: Model dose-response curves for the 2 test lines*

# 3. Analytical performance

## 3.1 Membrane optimisation

The concentrations of vancomycin antibody and streptavidin on each line had to be optimised to allow the conjugate to bind efficiently and to avoid false positive results. The sample of antibody was diluted to concentrations of 1 mg/mL and 0.5 mg/mL, and 0.4, 0.8, and 1.2 µL were pipetted per membrane line. The avidin was supplied in samples of 0.1 mg/mL and lines of 0.4, 0.8, and 1.2 µL were pipetted as well. The membranes were manually printed by pipetting spots of 0.1 µL in a line; for this technique, it was found that a volume of 0.8 µL yields a continuous line and minimises the issues of solvent spreading. The more concentrated antibody solution was chosen, as it gave more intense line colours.

To slow down the wicking speed the conjugate pads were soaked in an aqueous solution of glycerol. The ideal concentration was determined by testing 2%, 3%, 5%, 10%, 15%, 20% and 30% glycerol solutions. Whilst the higher glycerol concentrations led to a slower and more controlled migration of the conjugate down the membrane, they also prevented all the conjugate from departing from the pad. Thus, a compromise concentration of 5% glycerol was considered ideal.

After hand-printing the membranes with the antibody and streptavidin lines, they were soaked in a BSA-Tween-PBS solution to block all other binding sites on the membrane and prevent non-specific interactions between the conjugate and the membrane from occurring. The BSA concentration tested was in the 5% - 10% range and the soaking time was varied (1 min to 3hrs). Soaking times longer than 5 min eliminated random membrane-binding of the conjugate.

An unsolved issue at the moment of writing is the migration of the conjugate along the membrane. Whilst clearly binding to both of the test lines, the conjugate has difficulty travelling up the membrane and sometimes remains immobilised onto the nitrocellulose film. If future tests, in which the membrane soaking time/glycerol concentration/antibody concentration parameters are to be further optimised, fail to give better results, then the migration problem is most likely due to micro-defects caused by the inaccuracy of hand-pipetting the antibody/streptavidin solutions on the membranes. An elegant solution to this is employing a specialised fine printing machine, which would dispense the necessary volumes of compounds on the membranes without affecting the nitrocellulose film.

For now, the sensing device only works on blood plasma. However, implementing blood-separating membranes instead of using the classic nitrocellulose ones would allow for tests to be done directly on blood, without the need of prior separation of the sample. The Vivid™Plasma Separation Membranes produced by PALL offer a one-step plasma segregation from the whole blood and they have been shown to give the same plasma composition as the classic centrifugation method[4].

## 3.2 Calibrating the biosensor

Sensor calibration was done by testing blood plasma solutions with varying vancomycin concentrations (from 5 to 100 mg/mL) against the membranes. The app was used to measure the intensities of coloured lines against the background intensity. The colour intensity of the lines was proportional to the amount of conjugate bound to the antibody or streptavidin, the latter being proportional to the displacement by vancomycin. As the concentration of the drug goes up, the amount of conjugate bound to the antibody sample line decreases as it is displaced by the drug. The free conjugate then binds to the streptavidin so the colour intensity of this line increases as the vancomycin concentration increases. Therefore, the colour intensity of each line was measured against different vancomycin concentrations to create a calibration curve. This can then be used to detect the concentration of vancomycin in unknown samples by matching the colour intensity of the sample lines from the test with the calibration curve.

The app was tested on different mobile phones by analysing the same membranes in the same environments. It was found that the differences in intensity read-outs from phone to phone can be correlated to the screen resolution of each phone, so the app can be modified to account for screen resolution and show intensity results accordingly. The image results obtained with the novel app are confirmed by already available, widely-used software like ImageJ.

# 4. Novelty and Creativity

## 4.1 Already Available

The biosensor design was modelled around a few central ideas: it had to be quick, efficient, and straightforward to use. Ideally, the group wanted to create a device which could easily be used by patients at home, so that inconvenient visits to hospitals/clinics can be avoided. Therefore, the inspiration source was represented by already available biosensors on the market, as these were designed for home use. The well-known pregnancy test was a starting point in the research, because it meets all the requirements of our biosensor. Further research[5, 6] and consultations with Prof. Cass confirmed that a similar method could be employed in the biosensor. While some lateral flow assay readers exist currently, these are often large, unwieldy, table-based devices. The team wanted to take inspiration from these and aim to designing a more portable version.

## 4.2 New developments

The standard lateral flow pregnancy test, which contains a test antibody line (to detect the presence of hCG, confirming or infirming pregnancy) and a control line (to test whether the sensor is functional or not), gives only qualitative results. However, the biosensor needed to accurately quantify the concentration of vancomycin as well as detect its presence, so the technique was further improved by analysing the colour intensity of the lines and converting the intensities into drug concentrations.

A single test line, containing vancomycin antibodies, would not be enough to yield an accurate result; the competitive assay format leads to a signal intensity that is inversely proportional to the drug concentration and has a limited detection range (dictated by the amount of conjugate that is displaced from the analyte at the antibody site). These initial limitations have been overcome by employing a second printed line on the membrane, containing streptavidin molecules, which can bind the displaced conjugate through biotin-streptavidin interactions. Therefore, the intensity of the second line is proportional to the vancomycin concentration in the sample. Concentrations can be extrapolated from the analysis of both lines; this yields a greater accuracy, even at very low and very high concentrations.

SensImperial also aims to change the current perception of blood analysing machines by replacing the classic, expensive, bulky hardware devices with a miniaturised sensor: specialised LFA membranes and a companion smartphone app.

Using a phone camera, the biosensor has the equivalent of thousands of photosensitive sensors in the form of a CMOS sensor. The accuracy of large table based lateral flow assay readers can be achieved using nothing more than a smartphone camera and a companion application. This novel approach reduces manufacturing costs to essentially nothing whilst increasing the availability from tens of people in specialised laboratories to billions of people with smartphones.

The design can also be modified to detect other chemicals, using the same lateral flow assay design. This would require relatively few changes: replacement of a vancomycin antibody with a new suitable antibody for the analysed molecule and change of the vancomycin moiety on the complex with the new target compound; there is huge scope for development and innovation of this design, especially in healthcare. When implemented, this sensing system (membrane-smartphone camera-app companion) can lead to a great reduction in blood tests costs and a much better patient experience. When suitable for analysing multiple biomarkers' concentrations, this sensing system will be a much more affordable and sustainable alternative to currently used hardware devices, reducing acquisition, maintenance and training costs altogether, whilst providing quicker measurements.

Further improvements may involve porting the software to Apple's iOS as including encrypted cloud storage so that patients and doctors can interact with the results without having to schedule hospital appointments thus reducing hospital strain.

# 5. Translation potential

## 5.1 Stakeholder desirability

The current procedure for administering vancomycin relies heavily on tests that determine the blood concentration of the drug. After an initial loading dose is administered, the trough levels of vancomycin are measured 30 minutes before the next dose is due. Further doses and dosing intervals are adjusted based on this measurement. The frequency of monitoring depends on the patient's response to treatment[7]. This method raises several issues, impacting patients, hospitals and their staff, and private insurance companies.

First, patient monitoring can only be done in hospitals or clinics, since it requires large and expensive equipment. Our biosensor is small and portable; the software can be installed on any Android phone and each membrane measures 1 cm x 6 cm. This benefits patients, since the measurements can be conducted anywhere, including in their own homes. This would allow children, less abled or elderly patients to avoid inpatient treatment. A live-in or visiting medic or nurse could administer and monitor the drug, increasing the comfort of the patients.

Second, the current equipment requires hospital staff to have a certain amount of training and experience to perform the tests, as it requires specialised laboratory processing. In contrast, our software is straightforward and easy to use, with a user-friendly interface and built-in instructions.

Third, the financial aspect of implementing a new sensing device is a major consideration. Current tests are rather costly, regardless of whether the hospital, the patient or a private insurance company is paying for it. Our method is much cheaper than the current tests; the use of a software platform rather than a custom-made hardware device drastically reduces costs for all parties. Hospitals would not need to purchase expensive equipment, nor would they need to hire or train specialists to perform the analysis. Patients and private insurance companies will also benefit from the lower treatment costs.

The stakeholders involved in vancomycin administration would benefit from this specialised device. In the case of medical staff, the short test times, low costs and ease of use would lead to more efficient treatments. For patients, the experience would be more comfortable, allowing for outpatient treatment in certain cases and requiring minimal quantities of blood. The use of smartphones in hospitals has been increasing[8]. This trend is perceived positively by many physicians[9], as shown in the survey results attached in Appendix 1, and there are numerous instances where this practice has significantly decreased the duration of hospitalisation[10]. Thus, the use of a smartphone software platform has a place in the future of hospitals and has the potential to improve the quality of life for all involved. The pain relievers and gain creators are summarised in the table below.

| Stakeholder | Gain creators | Pain relievers |
|---|---|---|
| Hospitals & staff | <ul><li>Software-based</li><li>Great potential for expandability</li><li>Easy to update and improve the system</li><li>Small, portable</li><li>Scalable costs based on need</li></ul> | <ul><li>Nearly instantaneous results</li><li>No external (i.e. laboratory) processing required</li><li>Minimal / no training required</li><li>No hardware required</li></ul> |
| Patients | <ul><li>Easy access to one's medical data</li></ul> | <ul><li>Minimal amounts of blood required</li><li>Allows for outpatient treatment</li><li>Lower testing costs</li><li>Shorter hospitalisation times</li><li>Increased comfort</li></ul> |
| Insurance companies | <ul><li>Potential for partnerships with hospitals</li><li>Easy access to patients' medical data (with their consent); informed cost decisions</li></ul> | <ul><li>Lower costs per patient</li><li>Increased profits</li></ul> |

## 5.2 Technical feasibility

The use of a software platform rather than a hardware design means that rapid prototyping was easily carried out. The application is very simple to understand, and the interface is designed so that specialist training is not required. It is compatible with the Android operating system, so it can be easily run by over a billion people in every country without the use of specialised equipment.

Currently, there are no devices similar in size and abilities on the market for sensing this drug. Vancomycin analysis is done exclusively in laboratories, using specialised instrumentation (e.g. Abbott Architect, Roche Cobas c311/511, Beckman Coulter AU480)[11,12].

Our biosensor would not require any alterations to make it fit for the market. However, the manufacturing process would have to be scaled up. Membrane manufacturing can be scaled up by printing the antibody and streptavidin line on a large sheet of nitrocellulose membrane, using specialised printing equipment, and then cutting it down to size. The chemicals used to synthesise the conjugate are all non-toxic, while the de novo synthesis is relatively simple. Most of the solvents used are aqueous electrolytes and do not require special care, nor do they pose any threat to the environment. This means the procedure can be scaled up with little to no danger. The application code would need to be improved slightly and rigorous debugging and testing on more devices would need to be done.

To develop the sensor for commercialisation, the app can be released on the Android App Store where it can be downloaded and used by anyone. We will also work on a subscription service so that hospitals could pay annually for a software platform that would allow unlimited use of the application and the use of the separately purchased membranes. More features can be easily added as all that is required is an application update. Further improvement plans include launching the app on the iOS smartphones as well.

## 5.3 Business Viability

Most of the profit for the biosensors will be obtained from the sales of the single use membranes, or in the case of hospitals, by using a subscription-based service. This consists in an annual payment that covers the costs of the application, and includes technical support, free software updates and access to lower cost membranes. The application profits will be complemented by the purchases of the membranes, dependent on the frequency of use of the biosensor. This will provide a steady and reliable revenue stream which will amount to an overall high profit over time. This equivalent of the 'Bait and Hook' model is beneficial to our business because it reduces the otherwise high customer acquisition cost, while generating profit from a continuous low churn rate from the consumable membranes.

The cost of manufacturing membranes is low; creating the application did not require any supplementary costs, and many people already have compatible smartphones, so the production of expensive hardware for commercialisation isn't necessary.

The current cost to produce one membrane is 53p (Appendices 3 and 4), and the envisioned selling price for one membrane is £1. Purchase of packs of multiple membranes would lower the acquisition costs down to 85p per membrane. The app subscription plans will be priced based on concurrent user licenses, so that they are tailored to the size of each hospital purchasing our sensing system. In the United Kingdom, the NHS would cover part of the costs; in other countries, these costs would be covered by their respective healthcare systems. Our profit projections for a year is estimated to be between £2,019,200 and £2,028,200 (Appendix 2), based on the number of patients requiring vancomycin treatment a year in England[13] and the potential number of hospitals purchasing our app. Since our young team of researchers does not have an enhanced entrepreneurial experience, we plan on collaborating with a specialised consulting company (e.g. EY Consulting), which would aid us in successfully launching and maintaining our biosensor on the market.

Our design benefits from its novelty but also its potential to expand towards analysing other biomarkers, which would increase the selling potential for our sensing device. In the case of future competition, we will be able to maintain control over the market by adjusting the prices of our membranes and licenses, whilst continuously improving our application to stay ahead of other designs.

# 6. Team and support

## 6.1 Contribution of the team members

Our team comprises ten undergraduates from various faculties across the College, including the Departments of Chemistry, Bioengineering, and Electrical and Electronic Engineering. As the youngest team in the competition, and with little background in biosensor technology, the Sensus competition was a challenge for us. However, after months of hard work, dedication, and determination, we can proudly celebrate the skills and abilities we have gained.

Due to the wide range of skills and expertise shown by our members, we have been successful in combining different aspects of synthetic chemistry, biotechnology and engineering to create our biosensor. Several sub teams, listed below, worked in tandem, focusing on individual aspects of the design. Ana, Diana, Charlotte, Josh and Peter were also in charge of the translation potential assignment, Ben and Josh created the SensImperial website, and Diana filled the project manager role.

| Sub team | Team members | Roles |
|---|---|---|
| Chemistry | Diana Berheci<br>Ana-Maria Dobre<br>Federica Raguseo<br>Carol Tock<br>Charlotte Trott | Documentation and research<br>De novo synthesis of BSA-biotin-vancomycin conjugates<br>Biochemical assays<br>Membrane printing<br>Physical and chemical analysis |
| Bioengineering | Agnese Grison<br>Edoardo Occhipinti | Colorimetric analysis of membrane<br>Creation of biosensors |
| Electrical Engineering | Joshua Jennings<br>Benjamin Nevett-Farman<br>Péter Udvardi | Design and creation of biosensor<br>Programming<br>Colorimetric analysis of membrane<br>Designing neural networks |

## 6.2 Support

SensImperial would like to give thanks to the numerous staff and supervisors who have supported our projected and helped our design come to life. In particular we thank:

- Professor Tony Cass, our project supervisor, who provided us with valuable advice and references, laboratory and work space, chemicals and consumables.
- Dr Thao Le and Dr Hanadi Hassan-Nixon, who taught us specialised laboratory techniques, purchased chemicals and equipment, and helped us with suggestions and advice throughout our research.
- Imperial College staff from the Chemistry and Electrical & Electronics Laboratories who have allowed us to use equipment and lab space, whilst giving us helpful tips.
- Dr Paul Wilde, Rebecca Middleton, and Philip Power, who have helped us obtain funding to cover living costs for the duration of the project.
- Rufus Mitchell-Heggs, former SensUs Imperial participant, who has given us invaluable tips and advice on how to maximise our potential, based on his experience with the competition.

## 6.3 Sponsors

We would also like to thank our sponsors who have backed the team financially. Without their help, the project would not have come to life.

- The Imperial College Dean's Fund, which provided us with the necessary funds to afford living in London during the summer when most of work on the biosensor was undertaken.
- Bio-Nano-Consulting, who have contributed financially towards our team.

# 7. Final Remarks

This project has presented new challenged to our team and provided various opportunities to learn new technological and transferable skills. Our team has gained experience in many areas outside our proficiency which will be invaluable to future endeavours. SensImperial would like to thank all the people who have contributed their time and energy into the project. Without their support, our project would not be the success it has become.

Looking to the future, our team would like to further improve and optimise our biosensor, to make it ready for commercialisation. We firmly believe our idea is both innovative and feasible, but due to lack of time, we haven't been able to fully calibrate and refine our apparatus. We plan on finishing all the remaining steps and launching our biosensor on the market by the end of the next academic year. We strongly believe that the downsides of this design are minimal and can be further reduced by implementing the improvements mentioned in the previous sections. Moreover, we have already received enthusiastic feedback on our work from medical staff around London hospitals and we have been offered the possibility of piloting our sensing system across hospital settings.

One of the best parts of our idea is that our design can be adapted to test for other antibiotics or small molecules as well, the only required change being replacing the anti-vancomycin line with a specific one for the new tested antibiotic / small molecule. Therefore, our vancomycin biosensor is only the first step towards quick, personalised healthcare and will easily enable painless drug monitoring for a large range of small molecules.

We want to thank SensUs for this incredible opportunity and we hope that the combined efforts of all the competing teams will lead to better worldwide healthcare opportunities.

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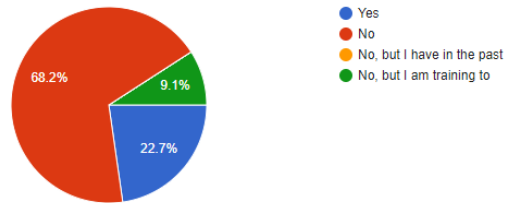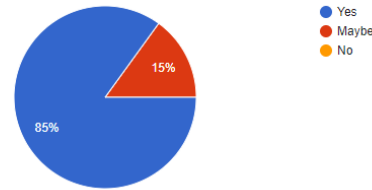
# Appendices

## Appendix 1 – Survey Results

SensImperial have run a survey regarding vancomycin treatment and monitoring within the Medicine Faculty of Imperial College and its associated hospitals; 44 responses have been gathered and the results are shown below:
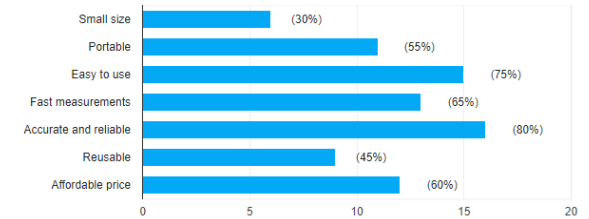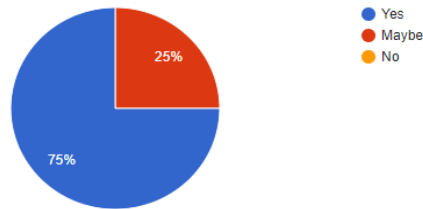
1. Do you work in the healthcare industry?
- Yes — 22.7%
- No — 68.2%
- No, but I have in the past
- No, but I am training to — 9.1%

3. Based on your experience, do you think an antibiotic monitoring device would be useful?
- Yes — 85%
- Maybe — 15%
- No

4. What features do you think are most important for such a device?
- Small size (30%)
- Portable (55%)
- Easy to use (75%)
- Fast measurements (65%)
- Accurate and reliable (80%)
- Reusable (45%)
- Affordable price (60%)

5. Would you use / purchase / recommend such a device?
- Yes — 75%
- Maybe — 25%
- No

6. As a patient, have you ever required antibiotic treatment?
- Yes, outpatient treatment — 54.5%
- Yes, inpatient treatment — 9.1%
- Yes, inpatient treatment that required close monitoring — 9.1%
- No, but I know someone who has — 22.7%
- No — 9.1%

7. Do you think an antibiotic monitoring device would have improved your experience?
- Yes — 59.1%
- Maybe — 13.6%
- No — 13.6%
- Yes, definitely — 13.6%

## Appendix 2 – Profit projections for one year

| Year 1 | Amount | Cost per unit | Selling price per unit | Profit per unit | Total profit |
|---|---|---|---|---|---|
| App subscriptions (per hospital) | 200 | £0.00 | £10,000.00 | £10,000.00 | £2,000,000.00 |
| Cartridges (membranes) | 60,000 | £0.53 | £0.85 to £1 | £0.32 to £0.47 | £19,200 to £28,200 |
| | | | | Overall Profit (per year) | £2,019,200 to £2,028,200 |

## Appendix 3 – Cost of components

| Compound | Supplier | Cost | Quantity |
|---|---|---|---|
| BSA (Bovine Serum Albumin) | Sigma Aldrich | £1123.00 | 100g |
| NHS-LC-Biotin | ThermoFisher | £159.53 | 50mg |
| Vancomycin Hydrochloride | Sigma Aldrich | £114.00 | 250mg |
| Vancomycin Antibody | BioRad | £155.00 | 1ml (5mg/ml) |
| Gold Colloid 40nm | BBI Solutions | £44.87 | 20ml |
| Streptavidin | ThermoFisher | £119.91 | 1mg |
| 2-mercapto-ethanol | Sigma Aldrich | £24.30 | 10ml |
| Solvents | ThermoFisher | £318.01 | 10L |
| EDC | Sigma Aldrich | £48.50 | 1g |
| Nitrocellulose membranes | Sigma Aldrich | £8.91 | 1 sheet, 210mx297mm |
| Glass Fiber Pads | Sigma Aldrich | £1.00 | 1 sheets, 20x30 cm 10 pads |
| Sample Pads and Wicks | Sigma Aldrich | £18.10 | 100 strips, 1.7x30cm 2000 pads |
| Dialysis Membranes | Fisher Scientific | £304.95 | 15m |

## Appendix 4 – Costs per membrane

| Chemical | Quantities for 5mL batches | Cost per 5mL batch (£) | Conjugate cost per membrane (£) |
|---|---|---|---|
| BSA | 70 mg | 0.7861 | |
| VCM | 77.8 mg | 35.4768 | |
| EDC | 300 mg | 14.55 | |
| Biotin | 5.55 mg | 17.70783 | |
| Mercaptoethanol | 50 uL | 0.1215 | |
| Au | 15 mL | 33.6525 | |
| Dialysis membrane | 1m | 20.33 | |
| Solvents | 2L | 63.6 | |
| **Total chemical costs** | | 186.22473 | **0.37244946** |
| Membrane | | | 0.001 |
| Glass pad | | | 0.1 |
| Sample pad | | | 0.0181 |
| Antibody | | | 0.0248 |
| Streptavidin | | | 0.009592 |
| **Total per test** | | | **0.52594146** |