SensUs

Template for Team Results Document

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IC SensUs

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This document is a template for describing the results of your team. This document needs to be sent as a pdf document to contact@sensus.org, **no later than Wednesday 30th August at 12:00 CET (mid-day, Netherlands time).**

The document will be provided to the jury on Friday 1st September. It will be published on the SensUs website on Friday 8th of September.

For questions about this document, please send an e-mail to contact@sensus.org.

Summary for the SensUs website (max. 200 words)

The concentration of NT-proBNP in a blood sample can be used to clinically assess a patient risk of heart failure. Our biosensor offers a novel detection strategy for this protein, which explots conventional antibody sandwich assay and optical properties of magnetic microparticles in a flow cell[1].

The flow cell is composed of 3 vertical layers through which the sample is filtered. The first layer is a fiber glass membrane (1-micron pores) with immobilized detection antibodies bound to streptavidin magnetic particles (0.7-0.9 microns). The second layer is a nitrocellulose membrane (1-micron pores) functionalised with capture antibodies. The final layer is where the unbound excess antibody-magnetic particle complexes are collected. A rare earth magnet is at the base of the flow cell to increase the rate of diffusion by attracting anti-NT-proBNP-functionalised magnetic nanoparticles. The sample is added to the fiber glass, where NT-proBNPs bind to the detection antibodies, and passes through to the nitrocellulose membrane. The capture antibody then binds to the NT-proBNP, forming a sandwich and immobilising NT-proBNP-nanoparticle complexes. This layer is then removed from the flow cell and imaged using a USB microscope. The images are analysed by ImageJ to infer the concentration of NT-proBNP from the number of magnetic micro particles .

1. Biosensor System and Assay (max. 1½ page)

Our biosensor detects the presence of NT-proBNP, a marker of heart failure. Our design is based on an antibody immunoassay with an optical platform for detection. From this we devised a flow cell which was 3D printed. It consists of 3 groves and a section at the bottom to insert the rare earth magnet.

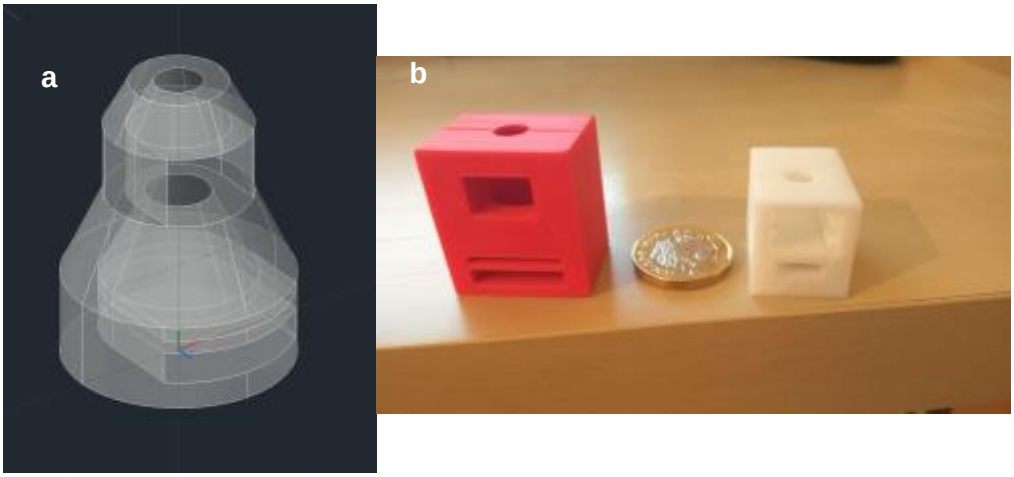

Figure 1. a) 3D Flow cell model in AutoCAD (b) Comparison between the conical flow cell (red, material: PLA) and the first rectangular version (white, material: ABS). Flow cell was made conical so the surface area was increased. The recangular outline was added to make it printable.

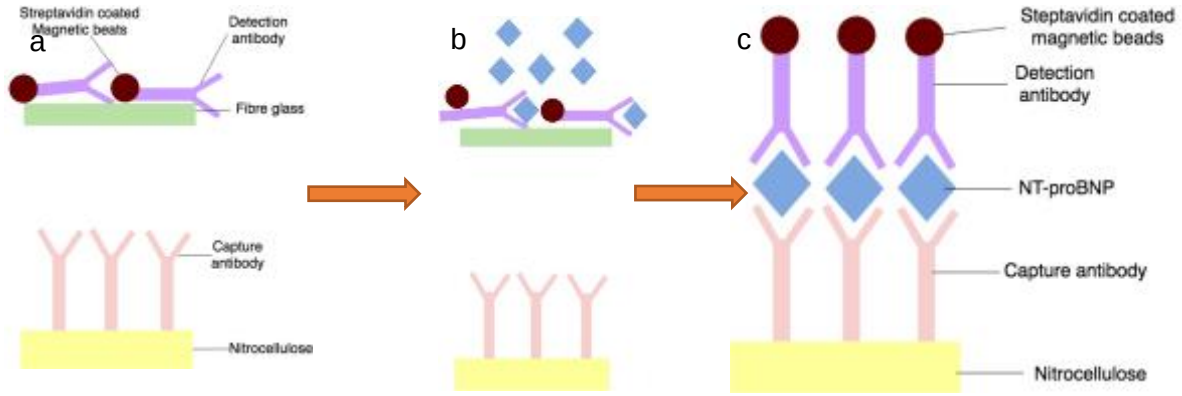

Figure 2. Schematic diagram of the operation of the sensor. (a) Idle sensor, with the fibre glass functionalised with the magnetic beads and antibodies adsorbed in the nitrocellulose membrane. (b) Addition of the plasma and flow of the complexes bead-antibody-antigen. (c) Close-up of the

This sandwich assay format requires 15C4 as the capture antibody and 29D12 as the detection antibody. We have incorporated a magnetic component in the form of 0.7-0.9 micron streptavidin magnetic particles which are bound to the biotinylated detection antibodies. A rare earth magnet is placed at the base of our flow cell to increase the rate of filtration and consequently the biosensors efficiency. The flow cell is composed of 3 vertical layers, the first being a fiber glass membrane (1-micron pore size) with the functionalized capture antibodies on the surface. The concept being that the detection antibody-bead complex bind specifically to the NTpro-BNP and this trimer would pass through the fiber glass to the nitrocellulose membrane (1-micron pore size) where the capture antibodies would recognize a second site on the NTpro-BNP and thus bind creating a sandwich.

The unbound detection antibody-magnetic bead complex would be collected on the final filter just above the rare earth magnet. The number of magnetic beads correspond to the concentration of pro-BNP in the sample. These beads are detected using a USB microscope. The picture captured from this microscope is linked to the image processing program, ImageJ which counts the visible clusters of beads. This Java based imaging program is a useful analytical tool that improves the accuracy and efficiency of detection. The data collected form the microscope and imaging software would be integrated by a code into an app thus providing the doctors with a coherent result. The microscope should be incorporated into the final device to contain all the components of the sensor therefore improving its portability.

2. Analytical Performance (max. 1 page)

To select the best antibody pair for our sensor we carried out an ELISA Sandwich assay with the provided antibodies. We established 15C4 and 29D12 were the more appropriate pair due to our absorbance results. We found a trend between the concentration on NT-proBNP and the absorbance, this was due to the presence of magnetic beads which only remained in the assay after the wash if they were part of the sandwich complex.

3. Novelty and Creativity (max. ½ page)

3.1. Already available

Heart failure detection may not be noticed by the patient during day to day activities until it reaches a more serious stage. One of the most common symptoms of heart failure is dyspnoea (shortness of breath), however congestive heart failure at times is not the only cause of dyspnoea. The rapid measurement of NT-proBNP in the blood easily prevents other causes of dyspnoea to be confused with heart failure[2] giving the patient a better prognosis. Sensors for NT-proBNP are already commercialized however none are tailored to NT-proBNP.

3.2. New developments

Our biosensor has progressed from our initial design as we have both encountered problems and come up with practical developments. At first, we were going to use gold nanoparticles as the detection antibody labels but decided against this because the particles were too small to be detected by the USB microscope, its maximum level of magnification is x300. Magnetic micro particles are both visible under the limited magnification of the microscope and the rate of diffusion is significantly increased by the attraction of the magnetic beads and the rare earth magnet consequently results are received more rapidly. The flow cell was originally in a cube shape but we altered this design to a conical shape because this increased the surface area of the membrane that was in contact with the sample. The diameter of the narrowest point is 6.5mm and this contains both the fiber glass and nitrocellulose membranes.

Originally, we were going to add the antibody-magnetic bead complex to the plasma sample then add this mixture directly onto the nitrocellulose layer. We opted to change this design and immobilize the antibody-bead complex on a fiber glass layer above the nitrocellulose because it meant the components of the sensor were kept together, increasing the commercialization of our product.

One of the key selling points for our biosensor is that there is no need for a wash to be carried out which is very common in immunoassays. Our biosensor would be sold with disposable filters which are easy to insert into the grooves and replaced after each use. The filters could be commercialized thus available to order and slot into the grooves of the flow cell.

4. Translation Potential (max. 1½ page)

4.1. Healthcare application potential

Our biosensor aims to optimize the detection of NT-proBNP competing with devices already available on the market. We aim for our sensor to become a portable, handheld device which will benefit doctors. For patients, a blood sample will have to be collected and centrifuged which is a minimally invasive procedure. The time taken for diagnosis will be reduced to 5 minutes once the sample of plasma is in the device. At the moment, it would not be as efficient as the Samsung LabGeo10 which detects NT-proBNP levels directly from blood samples.

The attraction between the rare earth magnet and the magnetic beads increase the rate of diffusion of the sample inside the flow cell, this reduces the detection time, improving the efficacy of our sensor. Additionally, various tests can be carried out requiring only the replacement of the disposable filters which will be sold alongside the product.

As the cut-off point for NT-proBNP in the detection of heart failure varies significantly with age difference (450-1800pg/ml), a large range will be necessary.

4.2. Industrialization and commercialization potential

5. Team and Support (max. 1 page)

5.1. Contributions of the team members

Aoife Keane – ELISA assay, purchase of supplies and other lab procedures

Maria Medeiros – ELISA assay, general lab procedures and literature review

Qien Li – ELISA Assay and general lab procedures

Vanessa Ho – Organisation and communication.

Ekaterina Pchelintseva – Functionalisation of the antibodies and general lab procedures. Literature review and research.

Amparo Güemes Gonzalez – Design of the web page and literature review and research.

Ignacio Medina Fernández – Design of the flow cells in CAD and literature review and research.

Akashaditya Das – Literature review and research and purchase of the supplies.

David Bazaga Garcia – Literature review and research.

Caoimhe Canavan – General lab procedures

5.2. People who have given support

Dr Tony Cass provided guidance regarding the production of the prototype.

Thao Li, has provided many tips and techniques on how to carry out some of the procedures to ameliorate our sensor.

5.3. Sponsors

6. Final remarks (max. ½ page)

References

1 Shen, W., Tian, D., Cui, H., Yang, D., & Bian, Z. (2011). Nanoparticle-based electrochemiluminescence immunosensor with enhanced sensitivity for cardiac troponin I using N-(aminobutyl)-N-(ethylisoluminol)-functionalized gold nanoparticles as labels. *Biosensors and Bioelectrics*, 18-24.

2 Maisel, A., Krishnaswamy, P., & Nowak, R. M. (2002). Rapid Measurement of B-Type Natriuretic Peptide in the Emergency Diagnosis of Heart Failure. *The New England Journal of Medicine*, 161-167.