SensUs 2019

Rheumatoid Arthritis: Adalimumab

"Managing Rheumatic Disease, by measuring with ease."

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1. Summary

This document shows all aspects of the Glasensor biosensor inclusive of our novel concepts and aspirations.

Our biosensor uniquely utilises a single technology to produce a range of effects. This is very different to other systems that usually require separate technologies to solve issues such as pumping and transduction. We explain as fully as possible the principles behind how we have managed to manipulate and direct heat throughout the cartridge design to develop our lab-on-chip concept in section 2.This also includes a look at how we solved some of the issues we encountered along the way with subsequently manipulating and directing liquids in various stages of thermal excitation between both the liquid and gas phases.

The use of a single technology within a single system translates into reduced production costs. The benefit of reducing the number of technologies leads to a reduction in the total cost per unit; something that wouldn't be possible if we required multiple technologies. Further details can be found in section 5.

This development will in turn will hopefully enable us to lower the overall cost of the product, ultimately making it a point-of-care system that is more accessible to clinics and healthcare providers.

2. Biosensor System and Assay

2.1 Molecular Recognition and Assay Reagents

Our assay optimises a capture molecule (HCA204, Biorad) specific to adalimumab (ADL) conjugated to a glass reaction surface. The principles of the molecular recognition within the Glasensor assay can be found in figure 1A. As illustrated in stage 1, HCA204(blue) is able to pull ADL from sample due to an affinity constant (K_D) value of 0.06 allowing for high specificity. In stage 2 the washed sample is then subjected to a rapid heating and cooling process to initiate unwinding of the protein structure enabling access to the thiols for tagging as shown in stage.3. This occurs across the reaction site (X) as indicated in the simplified schematic of the chip design shown in figure 1B. Once immobilised, the ADL-HCA204 conjugate is washed with a buffer (e.g PBS) released from the buffer reservoir (A). This disrupts surrounding free-floating serum proteins and cleans the sample leaving only the immobilised ADL before introducing the probe monobromobimane (MBBr) to the system. MBBr suspended in the anhydrous buffer (e.g.DMF/DMOS) is released from reservoir (B) and allowed to incubate with the reaction surface. The accessible thiols react with the non-fluorescent MBBr, displacing the bromine and creating a fluorescence which can be detected with ex.394nm/ em.490nm.

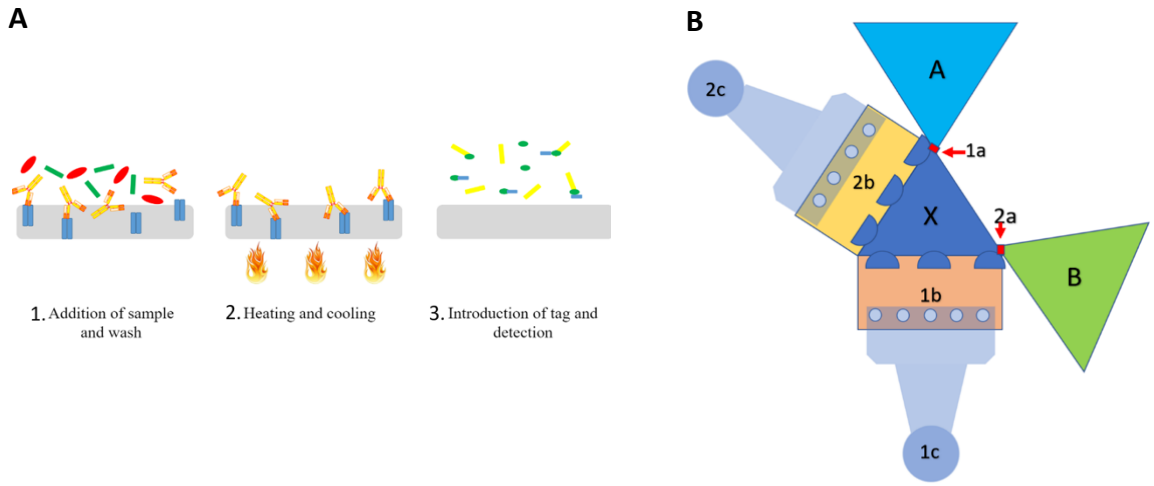


Figure 1: Glasensor assay. (A) Outline of principles of Glasensor assay. Once the sample has been bound to the active surface, residual proteins are washed away before the reaction site is rapidly heated and cooled to promote unwinding and denaturation of both the surface protein and the conjugated ADL. Unwound protein fragments expose thiol groups allowing for the introduction of the probe MBBr which gives off fluorescence upon alkylation of thiols. **(B)** Schematic of the cartridge. The main reaction and reagent regions are indicated; reaction site (X), buffer reservoir (A) and thiol tag reservoir (B). The associated waste channels (1b & 2b) with evaporation areas (1c, 2c) and valves (1a & 2a) are also shown

2.2 Physical Transduction

Our primary sensing system is optical utilising a photodetector to detect the fluorescent thiol-dimane conjugate that is produced once MBBr has alkylated the exposed thiol groups of ADL. These conjugate products give a fluorescent emission at wavelength ~490nm when excited by a wavelength of ~394nm. The reaction surface is an activated glass slip which has been salinized and is coated in an adalimumab (ADL) highly specific mutant (HCA204). This will be mounted inside a dark enclosure to maximise the photodetector's ability to read the fluorescent output, positioned above X as shown in figure 1B. This

will be correlated with a premade curve relating the number of visible thiols with the ADL concentration.

2.3 Cartridge Technology

The cartridge consists of two triangular shaped reservoirs containing the wash buffer and tag respectively and two rectangular waste reservoirs. These are connected to a reaction site in which the sample is placed as shown in Figure 2. The volume of reagents held by the reservoirs are approximately 200µl.

Capillary forces are used to transport the reagents in the microchannels to the reaction site. Heating elements (not shown) are located under each waste reservoir and the main reaction site. A capillary flow can be generated when a microchannel is wettable, i.e when the combined contribution of its four walls generates a negative capillary pressure pulling the liquid inside the microchannel. [1] Negative potential in the walls of the channel are created by applying heat to the bottom of the waste reservoirs, using a Peltier device, and inducing evaporation of the liquid from the waste reservoir. This allows the reagents to continue moving across the channel via capillary flow. Flow rates achieved are in the range between 25 to 96nl per second. The design of the reaction site was changed to a circular shape in order to ensure equal distribution of the reagent within it. The cartridge will be made using heat resistant plastic, such as Polymethylmethacrylate (PMMA) or Polyethylene Terephthalate (PET), to withstand heating at approximately 100°C. This material allows for reduced cost of manufacturing and convenient disposal of the cartridge.

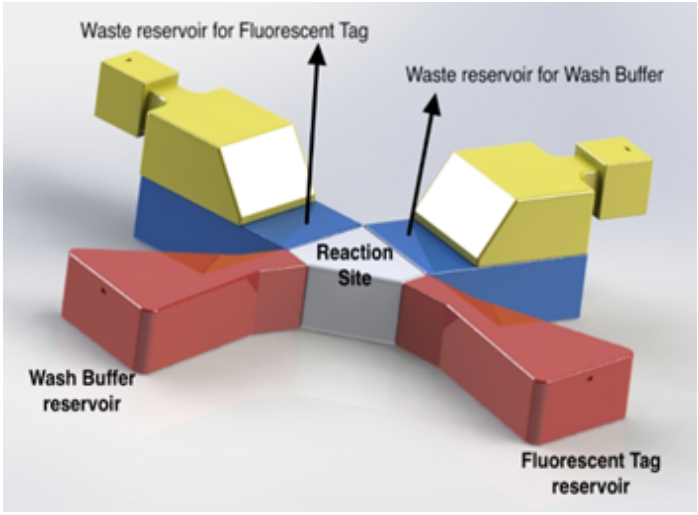


Figure 2: Glasensor Cartridge Technology Cartridge design drawn on CAD; reaction site, waste reservoirs, wash buffer and fluorescent tag reservoirs labelled.

2.4 Reader instrument and User Interaction

The reading instrument will be approximately 21 x 30 x 40cm in size and designed for bench top use. The temperature control system, power supply and detection system will be enclosed within this instrument. An LCD screen will be placed on the outside of the instrument to display instrument status and final results to the user. The one-use disposable cartridge will contain the required reagents enclosed within. The user will apply sample volume (~15ul) to the reaction site in the centre. and seal the cartridge with PCR film. The cartridge will be inserted into the instrument and secured in place by the user using a manual lever system. The user will then prompt the instrument to start analysis, an automated process, the final result will be displayed on the LCD screen. A full data folder can be transferred from the instrument via bluetooth for further, more detailed analysis of results.

3. Novelty and Creativity

3.1 Already Available

There are a number of technologies that influenced the final concept of our Glasensor lab-on-chip design spanning the 3 specialities within the team. In biochemical analysis companies such as BÜHLMANN have developed a Quantum Blue® Adalimumab system which is an in vitro diagnostic lateral flow immunoassay that utilises a miniaturized version of a sandwich ELISA to measure a colorimetric readout. There is also a recent biosensor in development that exploits thiols within IgG to sense copper ions from blood. However, in this process reducing agents were used to cause unfolding of the protein. [2]

In microfluidics, evaporation induced flow has already used to increase passive flow and pumping [1]. The use of evaporation to aid capillary action with passive pumping action to remove the waste from a chip and maintain a constant flow over long periods of time has also been characterised. [3] As well as adjusting the level of heat to control the speed of the flow using external electrical components (Peltier plates) to heat specific areas of the chip. [4]

In electronics, red, green, blue (RGB) sensors are widely used in the field of concentration detecting. Especially for those solutions in which the colour (RGB index) shifts proportionally as concentration changes [5] . Our temperature control system has also been influenced by a common structure of a microfluidics thermometer of Peltier plates connected with thermocouples. [6]

3.2 New Developments

Our team has utilised various existing techniques as outlined above to influence our novel, lab-on-chip design. This includes the use of a closed heating system to induce and maintain the flow of reagents and a unique rapid heating and cooling system to heat-shock the ADL sample into exposing the structural thiols as aspects we are very proud of.

Although evaporation induced flow has been well documented, it as yet hasn't been utilised to drag a buffer across the surface of the sample area to; wash impurities and remove waste. Using it in a double cross channel system where flow must be directed in different directions without crossing into the other channels has also been an interesting development. The time constraints of the competition required us to have fluids flow in short periods of time while allowing proper washing and tagging. Keeping the waste within the chip in a recessed waste chamber to avoid back-flow was also an innovation of the engineering team.

The electrical team has also optimised the use of Peltiers and thermocouples to relay a temperature by detecting micro-changes in the resistance of a fixed surface area. This creates a convenient, portable and accurate temperature control system which can switch between refrigeration and heating mode easily. We have also developed a user interface (UI) to communicate simultaneously with the temperature system and the detection system to display the relevant information to the user.

4. Analytical Performance

Quantification of Adalimumab using the Glasensor biosensor is dependent on the protein aggregation under heat stress and the effect this has on the concentration of exposed thiols. An anti-adalimumab antibody (HCA204) captures Adalimumab in the plasma sample to allow removal of contaminants through washing with a wash buffer. The correlation between Adalimumab concentration and decrease in concentration of thiols after heat stress was assessed.

Different concentrations of Adalimumab; 0, 200, 400 and 800µg/ml, were conjugated to HCA204 and underwent heat stress at 80°C for one hour. The thiol concentration was quantified using an Invitrogen™ Measure-IT™ thiol assay kit. As advised by the kit protocol, 5µl samples were added to 100µl thiol quantitation reagent. The samples were tested in triplicate and the line of best fit between the results was drawn, as shown in figure 3.

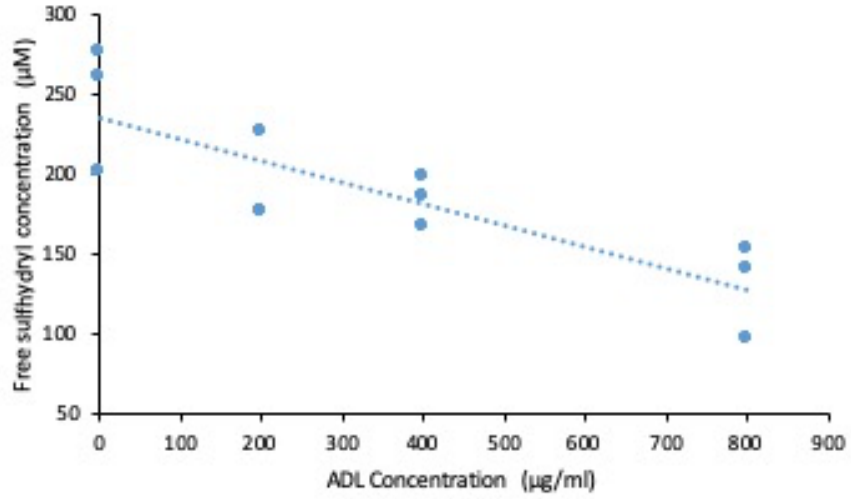
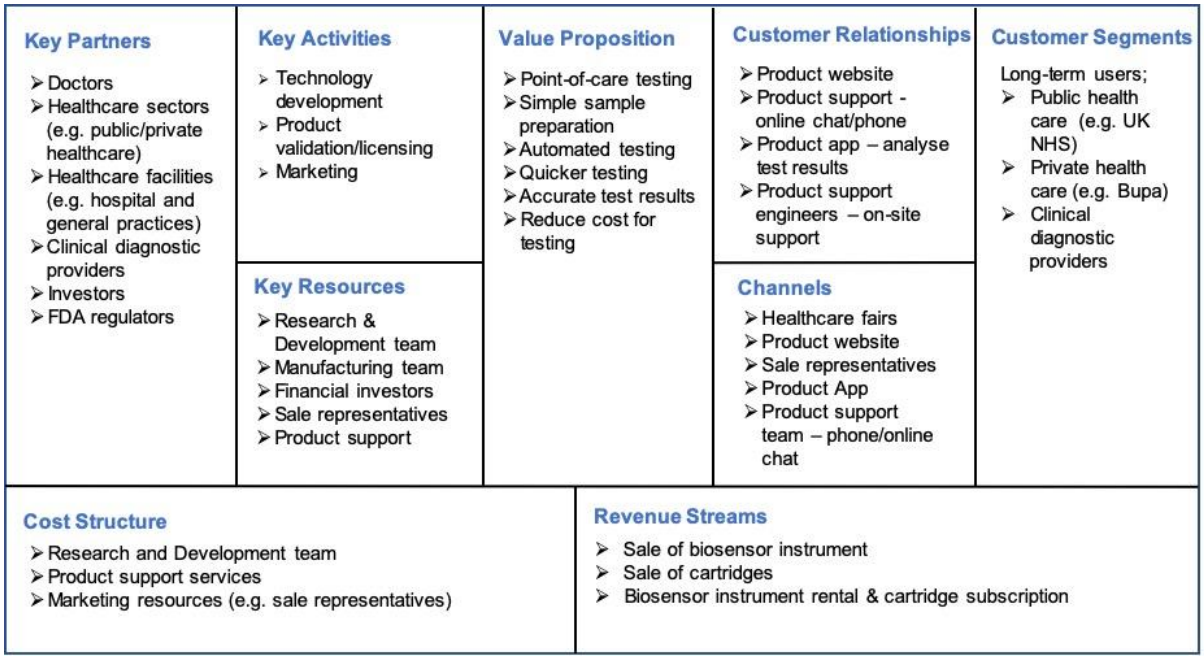


Figure 3: Correlation between concentration of thiol groups after heat stress and Adalimumab concentration. HCA204 was conjugated to 0, 200, 400, 800g/ml Adalimumab and incubated at 80°C for one hour. The concentration of thiol groups in samples was quantified by testing in triplicate (%CV = 6-14%). The trend line shows linear correlation (R^2 = 0.6964)

A negative correlation between the concentration of thiol groups after heat stress and concentration of Adalimumab was confirmed with a strong correlation coefficient of -0.8345. [7] Higher concentrations of Adalimumab were used to confirm a correlation due to the limited sensitivity of the thiol assay kit used. The thiol assay kit was unable to quantify the small changes in thiol concentration occurring at the required 0-10µg/ml Adalimumab concentration. The Glasensor biosensor is a microfluidic system that uses samples and reagents at least one order of magnitude less than that of the thiol assay kit. Further testing is being carried out to determine if reduced reaction volume and use of a highly sensitive fluorescent thiol tag can accurately quantify the change in thiol concentration at 0-10µg/ml Adalimumab.

5. Translation Potential

5.1 Business Model Canvas



5.2 Stakeholder Desirability

Rheumatoid arthritis affects around 400,000 adults aged 16 and over in the UK [8], 1.3 million Americans and as much as 1% of the worldwide population. [9] There is no cure for rheumatoid arthritis but the disease can be managed using anti-rheumatic drugs. Adalimumab (brand name Humira®) is an anti-rheumatic drug commonly used to treat rheumatoid arthritis and other autoimmune diseases (e.g. Crohn's disease and chronic plaque psoriasis). In 2018, Adalimumab sales approached $20 billion dollars with each patient typically costing $40,000 per year for Adalimumab treatment. [10] Adalimumab treatment requires regular monitoring of the drug concentration in the blood to improve effectiveness of the treatment and prevent adverse side effects such as drug resistance. [11] In the UK, current testing methods require samples to be sent to clinical laboratories and a turnaround time of 10 days.

The main stakeholders of the Glasensor biosensor are the public and private healthcare sector (e.g. NHS and Bupa), doctors and rheumatoid arthritis patients, as shown in figure x. The Glasenor biosensor provides a point-of-care biosensor to monitor the concentration of Adalimumab in Rheumatoid Arthritis patients. A disposable, easy to use cartridge design allows the healthcare provider to dispense a blood sample into the cartridge and insert the cartridge into the biosensor instrument for automated testing. Results are produced in minutes and shown to the user as the concentration of Adalimumab present in the sample. Additional data of the results can be exported from the instrument for further analysis either via Bluetooth connection or the biosensor app. The results can then be used shortly after testing to alter the patients Adalimumab treatment to increase efficacy. For the patient, this can be done within the sample doctor's appointment, therefore, avoiding having to return for an additional appointment.

5.3 Financial Viability

The main revenue streams of this business will be through the sale of biosensor instrument and cartridges. In the UK, currently over 46,000 patients in the NHS are prescribed Adalimumab [12] and require monitoring approximately every 2 weeks throughout their treatment plan which can last for 8 weeks to potentially 10 years for long-term treatment. [13,14] Therefore, in the UK alone, a minimum of 184,000 Adalimumab assays are performed at an average cost of £5 per test. [15] The sale prices of the Glasensor cartridge is determined using these figures to maintain financial viability of the product and provide a competitive price.

The sale price will be dependent on the demand of the consumer and the purchase plan selected. The business model uses different purchase plans to maintain a lasting relationship with the consumer and to secure financial stability for the business.

	Plan1	**Plan 2** (min. 1-year subscription)
Cost of biosensor instrument	£5000	£500 per year rental (includes free engineer support)
Cost of cartridge	£5 per unit	£3-5 per unit (dependent on length of subscription)

5.4 Business Feasibility

They key resources of this business include a research and development team, investors, product support, manufacturing team and sale representatives. Establishing reliable and cost-effective manufacturing of the biosensor and cartridges is vital to providing a high standard product and the viability of the business model. With a strong sales representative team and mass-manufacturer the business is able to establish revenue streams through building relationships with key partners such as doctors and the healthcare sector. Essential to maintaining a strong relationship with these key partners is providing product support for the use of the Glasensor Adalimumab biosensor.

Investors are essential to gather the capital required to build this core business operation. A strong business model for the Glasensor Adalimumab biosensor is vital to capture the interest of potential investors. However, a model to expand the business is essential to reassure investors that Glasensor is a strong long-term investment.

The business model for expanding Glasensor requires a research and development team to develop a product portfolio. Continued development of the Glasensor Adalimumab biosensor will help ensure the success of the biosensor in a competitive market. Identifying the demand and market for other point-of-care biosensors can provide research and development projects to produce future products. The continued advancement of existing products and the launch of new products provides a long-term business model for Glasensor to increase revenue streams and expand.

6. Team and Support

6.1 Contributions of the Team Members

Biologists

Elaine Ma (MRes Biomedical Sciences)

Elaine focused on providing proof of concept and developing the Adalimumab assay. She also contributed to the development of a business plan for Glasensor and provided key administrative support and guidance to the team throughout the project.

Krishan Sharma-McLachlan (MRes Biomedical Sciences)

Kris focused on providing proof of concept and further characterising Adalimumab thiol groups under heat stress. He also explored his interests in entrepreneurship sessions and was essential in providing support and guidance to the team throughout the project.

Biomedical Engineers

Daisy Ferraro (BEng Biomedical Engineering – 3rd year)

Daisy provided administrative support to the team and was a key contributor to developing the Glasensor business plan.

Rebecca Shepherd (BEng Biomedical Engineering – 3rd year)

Rebecca contributed greatly to the characterisation of evaporation induced flow and was the team's CAD designer; designing the cartridge and biosensor layout.

Ruvarashe Hungwe (BEng Biomedical Engineering – 4th year)

Ruva focussed on the characterisation of evaporation induced flow and developing this method in the cartridge design.

Kaya Üke (MSc Biomedical Engineering)

Kaya contributed to team discussion throughout the project and assisted the rest of the biomedical engineering team.

Electrical Engineers

Zimo Zhao (BEng Electronics and Electrical Engineering – 3rd year)

Zimo developed and built the heat system and provided expert support for the electrical engineering team.

Lujie Peng (BEng Electronics and Electrical Engineering – 3rd year)

Lujie developed and built the LCD interface for the biosensor instrument.

Zehao Zhang (BEng Electronics and Electrical Engineering – 3rd year)

Zahao developed and built the detection device to quantify fluorescent thiol tag.

6.2 People Who Have Given Support

Dr Julien Reboud (Senior Lecturer – Biomedical Engineering)

Thank you to Julien who provided guidance and encouragement to the team throughout the project. His support was essential to helping the team overcome difficulties throughout the project and prepare for the innovation days in Eindhoven.

6.3 Sponsors

BioRad

Thank you to Deneen Holohan (antibody specialist) at BioRad for taking an interest in Glasensor from the very start of the project and for providing a discount on the antibodies used in the Glasensor biosensor.

7. References

1 Temiz, Y., Skorucak, J. and Delamarche, E. (2014) Capillary-driven microfluidic chips with evaporation-induced flow control and dielectrophoretic microbead trapping. In Microfluidics, BioMEMS, and Medical Microsystems XII (Gray, B. L., and Becker, H., eds.), p 89760Y.

2 Chandra, S., Dhawangale, A. and Mukherji, S. (2018) Hand-held optical sensor using denatured antibody coated electro-active polymer for ultra-trace detection of copper in blood serum and environmental samples. Biosens. Bioelectron. **110**, 38–43.

3 Lynn, N. S. and Dandy, D. S. (2009) Passive microfluidic pumping using coupled capillary/evaporation effects. Lab Chip **9**, 3422.

4 Nie, C., Frijns, A. J. H., Mandamparambil, R. and den Toonder, J. M. J. (2015) A microfluidic device based on an evaporation-driven micropump. Biomed. Microdevices **17**, 47.

5 Supannarach, S. and Thanapatay, D. (2008) The study of using RGB color sensor to measure the Curcuminiods amount in Turmeric (Curcuma longa Linn.) and Zedoary (Curcuma Zedoarie Rose.) by comparing colors with HSL system. In 2008 5th International Conference on Electrical Engineering/Electronics, Computer, Telecommunications and Information Technology, pp 529–532, IEEE.

6 McKenzie, B. A. and Grover, W. H. (2017) A microfluidic thermometer: Precise temperature measurements in microliter- and nanoliter-scale volumes. PLoS One (Cao, B.-Y., ed.) **12**, e0189430.

7 Koo, T. K. and Li, M. Y. (2016) A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J. Chiropr. Med. **15**, 155–163.

8 Versus Arthritis. Rheumatoid Arthritis (RA).

9 Freeman, J. RA Facts: What are the Latest Statistics on Rheumatoid Arthritis? 2018.

10 Herman, B. Humira sales approach $20 billion. 2019.

11 Bendtzen, K. (2012) Anti-TNF-α biotherapies: perspectives for evidence-based personalized medicine. Immunotherapy **4**, 1167–1179.

12 NHS. NHS set to save £150 million by switching to new versions of most costly drug. Oct. 2018.

13 Adalimumab. Natl. Inst. Heal. Care Excell.

14 Leavitt, M. (2015) Ten-year Humira study at the halfway point. Natl. Psoriasis Found.

15 Laine, J., Puolakka, K., Jokiranta, S., Eklund, K. and Väkeväinen, M. (2016) Cost-effectiveness of routine measuring of serum drug concentrations and anti-drug antibodies in treatment of rheumatoid arthritis patients with TNF-α blockers. Biol. Targets Ther. 67–73.