

TEAM RESULTS DOCUMENT

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TEAM MEMBERS

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1. Summary for the SensUs Website

As engineers, the Sensing Barcelona Team stands on a common goal: to create an effective biosensor which will improve the quality of life of many.

It was concluded, after talking to some patients that had experienced treatment with Adalimumab, that behind the realisation of the device, there was a human aspect that had to be taken care of. Besides of being able to reduce notoriously the costs of the treatment, being able to quantify the amount of Adalimumab in a patient's body could reduce the painful supply process, avoid its rejection and assure the remission of the symptoms.

After an extensive study, the team considered several possibilities to tackle the challenge and decided that the best option was to opt for creating an optical sensor. Its main function would be quantifying numerically the concentration of the drug in a plasma sample by means of an image processing software.

Benefiting from the chemical and pigment properties of the colloidal gold molecule, characterized by its reddish colour, the device detects the intensity of the sample through image acquisition and associates it with a certain concentration. In other words, the intensity of the pigment is directly proportional to the amount of Adalimumab.

2. Biosensor System and Assay

The purpose of Sensing Barcelona's biosensor is to monitor levels of Adalimumab in human serum. This immunosuppressive medication inactivates Tumour Necrosis Factor alpha (TNF- α), the main cytokine that causes inflammation in joints. Accurate detection is important for the administration of the right dosage of this drug to each patient, due to the high interpatient pharmacokinetic variability. Sometimes, patients may develop autoantibodies against ADA that can influence on the effectivity of the treatment; ADA loses the capacity to inhibit TNA- α . In order to appropriately monitor not only the right dosage of ADA in serum, but also the body response of the patients to the treatment, this report presents a novel lateral-flow immunochromatographic strip.

Our lateral-flow strips provide rapid diagnosis to detect a wide range of samples without pre-treatment, with a low sample volume. These tests can be long-term preserved and are inexpensive. To make visual quantification easier, colloidal gold nanoparticles (red colour) have been added to the human serum sample. These nanoparticles are used as a reporter because of their stability, controllable particle size, and good compatibility with biological molecules.

Sensing Barcelona's LF strips are 4 mm wide and 5 cm long, consisting of 1 cm sample pad, 2.5 cm nitrocellulose and 2 cm absorbent pad; both sample and absorbent pad each overlap 2.5 mm with the nitrocellulose membrane. The nitrocellulose is attached to a plastic backing and is printed with the two main lines and a control line. One of the main lines contains TNF- α which binds to free ADA in the sample, the other line comprises F(ab) fragments of ADA antibodies for anti-ADA antibodies detection. Although common LF tests usually do without line control, our strips include this third line which contains ADA that picks up free colloidal gold in order to confirm the test has operated correctly.

As we can see in Figure 1, the lines have been printed in the nitrocellulose following a coherent order; T1 binds the free anti-ADA in the sample, T2 allows the quantification of ADA and, lastly, the control line will bind with the free colloidal gold.

The strategy of placing T1 as the first stage allows the user to be aware immediately if the patient is suffering an immunogenic response towards the treatment. If this line results positive the whole test becomes invalid.

T1. – Before printing the antibodies in the nitrocellulose, Adalimumab has been digested with Pepsin, an endopeptidase. This allows the obtention of ADA antibodies without the Fc part, since they are broken up during digestion. Only the Fab fragments of ADA are placed on the strip; Anti-ADA has high affinity for these Fab fragments and will bind to it. (Colloidal gold is bonded to Fc of anti-ADA)

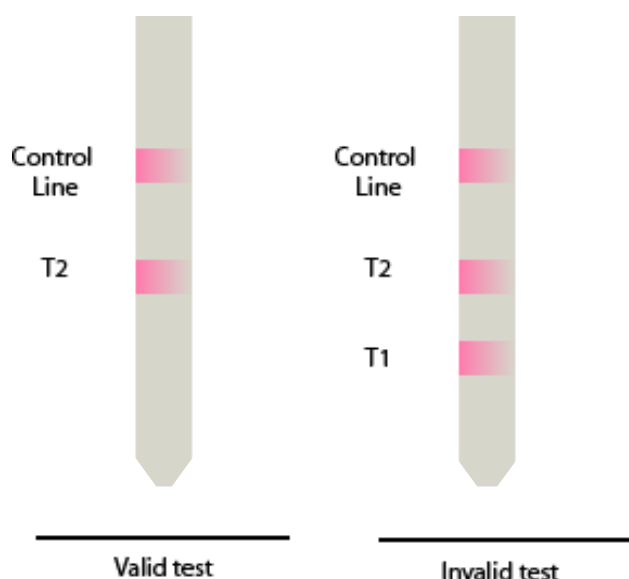


Figure 1 - Demonstration of a valid and invalid test

T2. – 0,1 nanograms/millilitre of TNF- α are placed over the nitro for quantification of free ADA. Fab of ADA binds to TNF receptor and colloidal gold binds to Fc.

Control Line. – Consists 100% of ADA antibodies.

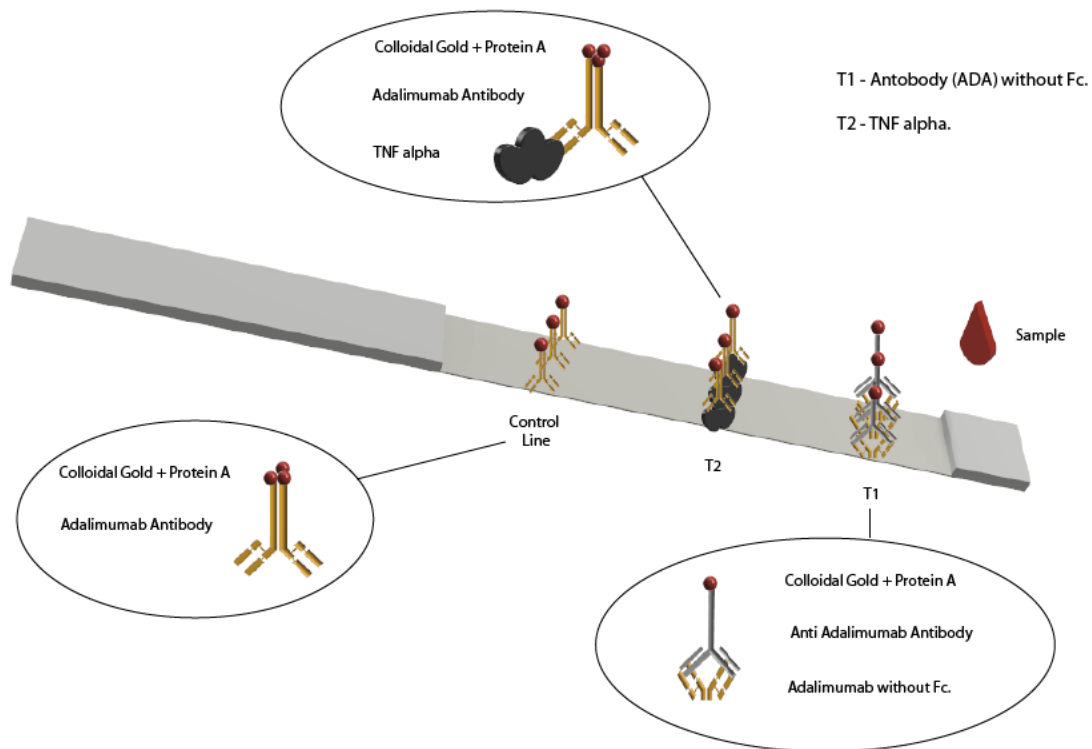


Figure 2 - Diagram of lateral flow assay. Three detection areas: T1 (Immunogenic Response Indicator), T2 (ADA quantification) and C (Control Line).

The assay consists of four sequential flow steps, that the sample pad has to absorb:

1. First, the “sample” is high salt lateral flow assay buffer (HSLF, 100 mM Hepes pH 7.5, 270 mM NaCl, 0.5 % (v/v) Tween-20, 1 % (w/v) BSA) spiked with 5 μ L ADA (ADA, 100 mM Hepes pH 7.5, 270 mM NaCl, 0.5 % (v/v) Tween-20, 1 % (w/v) BSA).
2. The second step is a wash step with 20 μ L HSLF, initiated immediately after the 45 μ L “sample” has been absorbed by the sample pad. The wash removes excess of antibodies to minimize potential interaction of the protein A with unbound antibodies.
3. In the third flow step, 50 μ L of reporter (45 μ L HSLF + 5 μ L protein A with colloidal gold) is applied to the strip. The colloidal gold is responsible for the conjugate’s bright red colour.
4. Lastly, there is another wash with 200 μ L of HSLF to remove excess of colour. This is important for the Lateral Flow Reader to work properly.

After the strip has undergone the sequential flow steps, it is ready for the quantification procedure. The strip is immediately inserted in the reader device developed by the Sensing Barcelona Team.

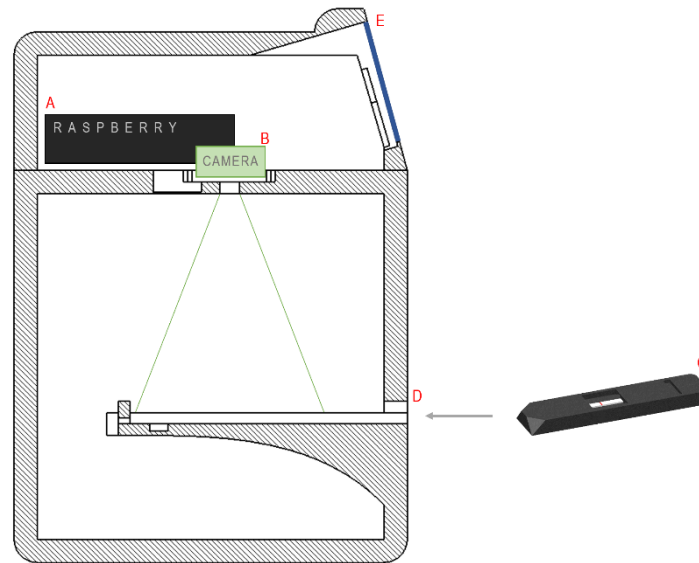


Figure 3. Cross section of the device that uncovers the Raspberry (A) and the camera (B). The cassette with the strip (C) is inserted through the opening (D) and subsequently a picture is taken and processed by the raspberry. The result is shown on the display (E).

In order to prepare the reader, a calibration program has to be ran and the strip has to be placed in its cassette. After this is done, the cassette is inserted into the reader. At this moment everything is ready for the quantification. Running the measurement program will deliver the quantity of ADA in plasma and the R^2 value at what the device is working. In order to do capture and process the image information the system uses a Raspberry Pi. The information is analyzed mainly with the support of ImageJ Software combined with Python scripts. All these features will be displayed in the 85.06x56.21 mm touchscreen.

The reader measures 230x170x170 cm.

In order to make the GUI more user-friendly and more visually attractive icons have been added and instructions are clearly displayed.

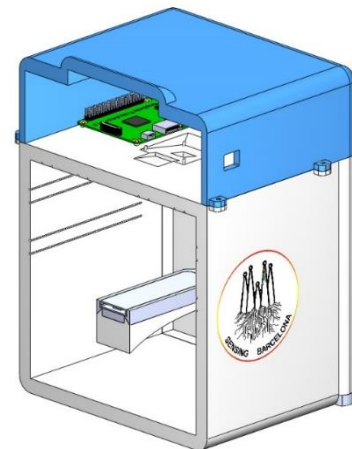


Figure 4 - CAD design of the device.

3. Novelty and Creativity

3.1. Already Available

- Lateral Flow immunoassays are widely used. Having Enzyme-Linked immunosorbent assay (ELISA) as one of the most commonly used. [1]
- The use of colloidal gold as a marker.
- Quantification of the sample through Image processing.

3.2. New Developments

In order to provide the user with the most efficient and reliable solution possible with have implemented innovative details throughout the whole process.

Firstly, our strips are shaped with a triangular tip in order to optimize the absorption processes during sequential flow steps. Because we use very small amounts of the samples it is of high importance that all of them are absorbed. We have found that re-shaping our strips makes sure that this takes place.

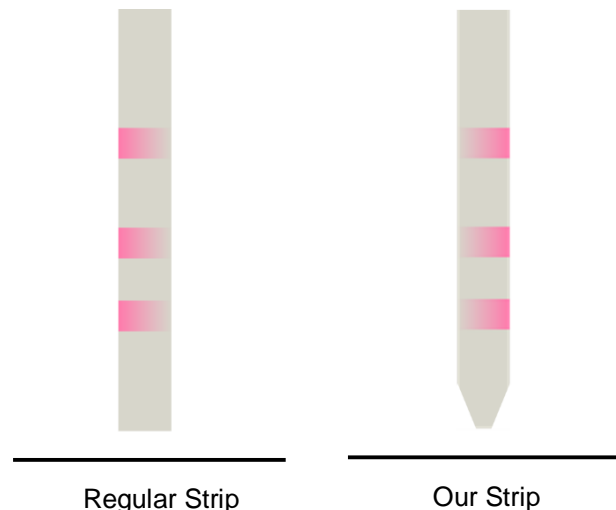


Figure 5 - Comparison between regular strips and our model.

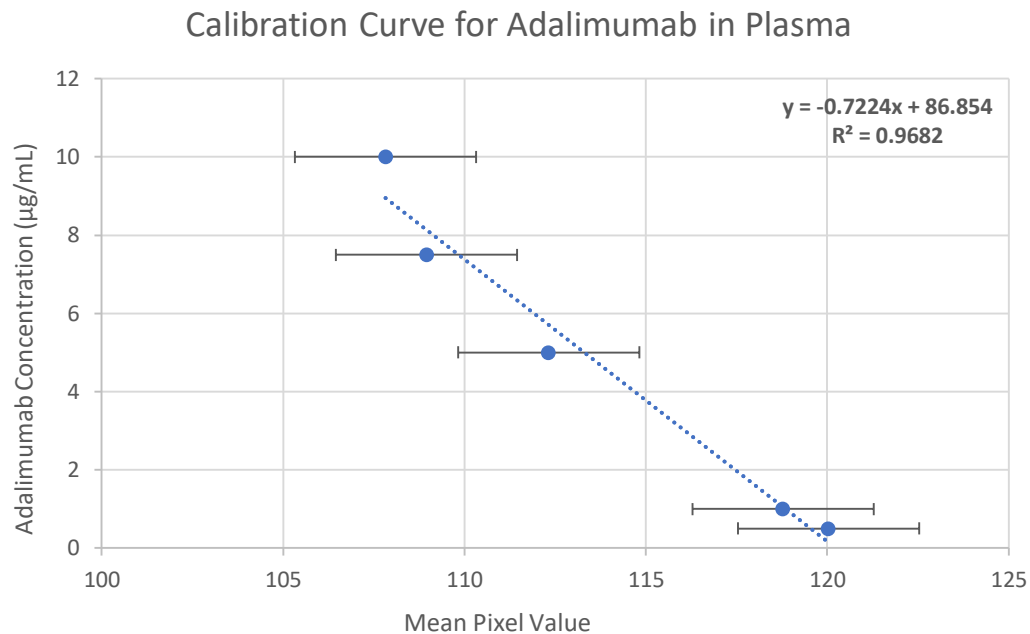
As mentioned previously, we have also reduced the quantities of samples used. This reduces the duration of the test. In order to continue reducing the duration of the test, the second wash is done with a large quantity (200 μL) of HSLF. This is not a common practice, but because the lower part of the strip has no relevant information, fully covering it with the wash doesn't affect the results and reduces the time that the HSLF need to get to the test lines.

Moreover, beyond creating a test only for the quantification of adalimumab in Plasma, we have also added a test-line which detects the presence of anti-ADA antibodies in the patient's serum. This information is of high interest to understand the patient's response to the treatment.

Lastly, we have been able to develop a simple, but robust, quantification system based on Python and the ImageJ Software. Creating Macros in ImageJ allows the automation of image analysis avoiding the need of having the user do it manually.

4. Analytical Performance

Doing all the procedure mentioned previously, takes approximately 15 minutes. Several slots of experiments were made during the development of the device. We always used the following known concentration of ADA in plasma: 0.5, 1.0, 5.0, 7.5 and 10.0 $\mu\text{g/mL}$. With the results of our device we obtained the following Curve for the quantification of ADA in Plasma.



As shown in the graph, we obtained results with a R^2 value of 0.9682 and a Trend Line defined as $y = -0.7224x + 86.854$, being x the Mean Pixel Value, calculated by our device and y the concentration of ADA.

5. Translation Potential

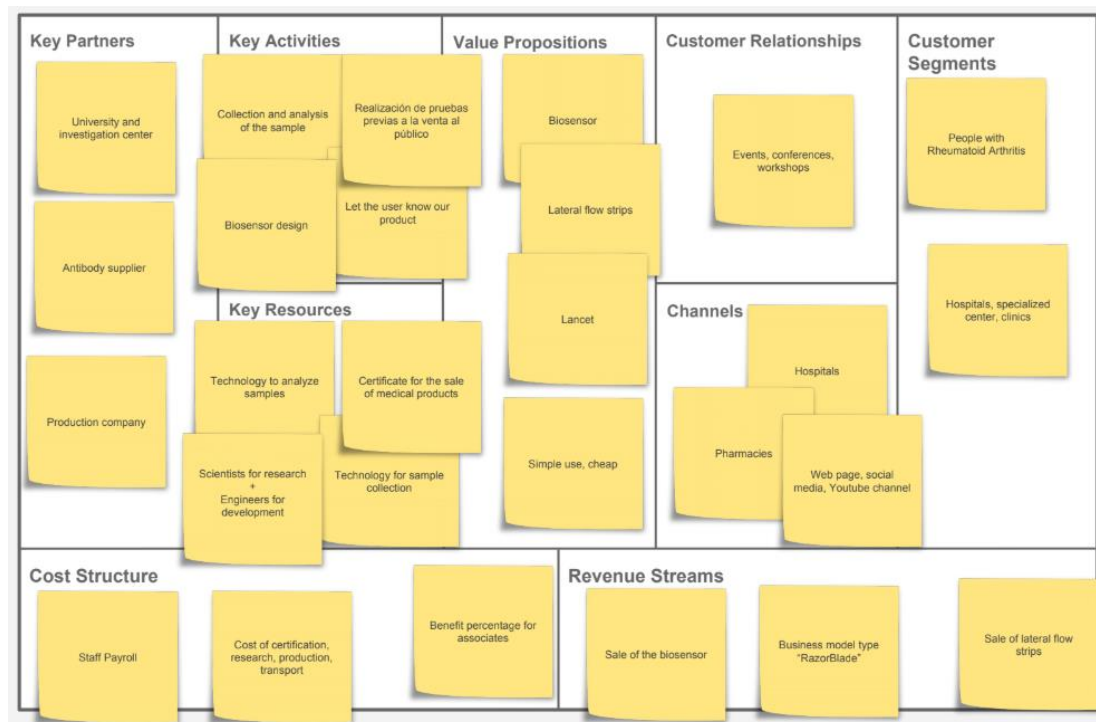


Figure 6 - Business Model Canvas.

Rheumatoid Arthritis causes swelling in the articulations which results in dysfunction of the joints, mostly in fingers and wrists. This is a chronic disorder. However, treatment is being administered to control the effects of the disease and prevent them from getting more severe. One of the drugs that is being used currently is Adalimumab (ADA). The administration of ADA needs to be accurate. If there is not enough drug, this disorder worsens, and if there is too much, the patient can build up tolerance to the drug. Periodic monitoring of ADA levels in the blood stream is key to prevent any of these effects. Currently, in the market, there are no devices which offer a quick solution for the quantification of ADA in blood. The current procedure takes at least 2 days, making it ineffective and a burden for the patients.

Only in Spain, there are about 200,000 people affected with Rheumatoid Arthritis, and each year, this number increases by 10,000-20,000 new cases [2]. These statistics are the motivation behind Sensing Barcelona for the development of a more effective solution.

Our device facilitates quantification of ADA by reducing the time-to-result to less than 20 minutes, allowing a doctor to monitor several patients daily. By obtaining the information quickly, the doctor has more time margin to adapt the treatment. Moreover, our sensor cuts medical costs by reducing the number of visits needed for monitorization and by making sure that ADA is administered only when is crucial. Minimizing test time also cuts costs by substituting 2h lab analyst work with 15 min of doctor's work which cuts costs from 30 to 5 EUR. As a further step, we included in our equipment a test line to analyze the body's response to the treatment – this way the doctor can control if the patient is having an immunogenic response to ADA.

Our team brings two new products to the world: The reactive lateral flow strips and a biosensor. Every characteristic of this device has been designed having patients and healthcare professionals in mind. That is why we have designed an intuitive and efficient device.

In order to analyze the financial viability of our product we have calculated a cost projection. We need to consider main groups of expenses First, the strips and dissolutions. Second, electronic instrumentation and 3D printed (PLA) case. The calculated final prices are 125,01 € for the biosensor and 29,65 € for the pack of strips. The cost of making the strips is a total of 19,1 €/pack, so we have a benefit of 10,55 € for each pack we sell. If we speak about the biosensor, its production costs 86,66 €, so our final benefits are 39,35 €. This adds up as 49,90€ of benefits per each complete pack sold.

Having in mind that the cases of Rheumatoid Arthritis around the world are increasing, the necessity of a device like ours is guaranteed.

For its commercialization two routes have been taken into consideration: On one hand, the current prototype will be sold for its use by professionals of the health-care system. For the monitorisation of several patients simultaneously. Need to be used with a centrifugation machine. And, on the other hand, a future prototype will include a microfluidic canal with an integrated separator [3], to avoid the use of the centrifugation machine.

As a first step, the current prototype of the se sensor would be introduced to hospitals in Catalonia, where the Sensing Barcelona Team is based. We are lucky to be established in the region with more hospitals in Spain, a total of two hundred thirteen in Catalonia from the seven hundred eighty-eight that are in the rest of the country. Most of them are private, this makes the introduction of our product easier, because there is less legal procedure and decisions are made by a fewer amount of people. Knowing this, we have estimated, in the first year, to sell between five hundred and a thousand unities of the biosensor, with a regular buying of the lateral flow strips increasing for each biosensor sold.

As a starting point, sales will be focused in hospitals with the rheumatology specialization. The percentage of hospitals with this type of specialization is about 30%, a total of 260 centres. Estimating that in the first iteration, only 40% of them will buy our product, that leaves us with 106 hospitals, with an average of two or three devices per hospital, plus the constant buying of lateral flow strips. Transportation costs will be charged to the buyer.

Setting us in the worse-case scenario, we would sell 500 units, which would add up as a total revenue of 43.330 EUR and a total benefit of 19.975 EUR during the first year.

The second year, we will introduce the home-version of the sensor to the market. Opening the doors to a broader market. Only in Catalonia, there are 37000 cases of rheumatoid arthritis, this translates as 37000 potential clients for our device.

Five years later from launch date, with a clearer idea of the market, we expect to expand our products to the rest of Spain and Europe, where the 1% of the population suffers from RA [4].

6. Team and Support

6.1. Contribution of the Team Members

Adao, Jahn Carlo: Worked in the Hardware development of the device.

Angosto, Ramon: Worked on the business case and feasibility of the device.

Benítez, Daniel: Worked on the software and the hardware development.

Escalera, Alex: Worked of the hardware department of the device.

Gallego, Helena: Designed the team's banner. Worked on various administrative tasks.

García, Oriol: Worked on the business case and feasibility of the device.

Mansilla, Laura: Designed our information triptych.

Martin, Maria Cristina: Worked on the development of the Sequential flow steps. Team Coach.

Mestre, Carlota: Worked on the development of the Sequential flow steps.

Moragues, Ignacio: Carried out support tasks in the software and hardware departments of the device. Worked on various administrative tasks.

Priddey, Nicole: Worked on the development of the GUI.

Remón, Paula: Worked on the software and the hardware development. Worked on various administrative tasks. Team Captain.

Solà, Josep: Designed the team's banner. Worked on various administrative tasks.

Tersol, Aina: Worked on the development of the GUI.

Villalba, Alejandro: Worked of the hardware department of the device.

6.2. People Who Have Given Support

Josep Ferre, Lab Technician at DFV

Vicenç Font, Operations director of IUL.

Patricia Planas, clinical psychologist and coordinator of the Catalan Rheumatology Association.

Laly Alcaide, director of the National Arthritis Coordinator (ConArtritis). She also suffers from arthritis and has taken Humira.

Dr. Ferran J. García Fructoso, Rheumatologist and founder of the Ferran Institute of Rheumatology (IRF) of Cima Hospital.

Tànit Tubau, patient who suffers from Chron's disease. Has taken Humira.

Paco Bogoñez, Professor at UPC. Helped us with hardware aspects of the device.

Jordi Solé, Professor at UPC. Supported us with administrative tasks at UPC.

Andrea Fernandez, Student at UVic. Administrative support.

6.3. Sponsors

Group Divasa-FarmaVic (DFV): Offered support in the sequential flow steps. Supplied the team with the strips needed for the testing and the competition.

IUL: Offered support in the prototype design. The 3D printed our model.



7. Final Remarks

This year has been a great learning process. Facing challenges, setbacks and successes as a team has showed us how important is to believe in our project. We have found that constant work and good communication are key elements to develop a product that makes us proud.

This wouldn't have been possible without the guidance of our supervisor Professor Jasmina Casals-Terré, our coach Cristina Martín and our sponsors, to you, we say: thank you!

Hasta Pronto,

Sensing Barcelona

8. References

- [1] J. P. Gosling, "A decade of Development in Immunoassay Methodology," *Clin. Chem.*, vol. 36, no. 8 Pt 1, pp. 1408–1427.
- [2] ConArtritis, "Todo sobre artritis - ¿Qué es la artritis reumatoide?" [Online]. Available: <http://www.conartritis.org/todo-sobre-artritis/que-es-la-ar/que-es-la-artritis-reumatoide/>.
- [3] H. Madadi, J. Casals-Terré, and M. Mohammadi, "Self-driven filter-based blood plasma separator microfluidic chip for point-of-care testing," *IOPscience*, vol. 7, no. 2, 2015.
- [4] J. Simmons, "RA Statistics," *RheumatoidArthritis.net*. [Online]. Available: <https://rheumatoidarthritis.net/what-is-ra/ra-statistics/>.