Team Results Document
BioLinkLx

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SensUs

Table of Contents

1. Abstract

Our team at the University of Lisbon has developed *AptaSense*, an innovative biosensor for continuous creatinine monitoring. This device uses aptamer-based sensors for high sensitivity and specificity in detecting creatinine levels. *AptaSense* integrates a biological recognition element with an electrochemical transducer, where anti-creatinine aptamers selectively bind to creatinine, and the transducer detects current changes correlating with creatinine concentration.

Featuring a 3D-printed microfluidic cartridge for easy sample handling, *AptaSense* is user-friendly and non-invasive. The initial results are promising with a clear calibration curve. The device includes a computational interface that displays creatinine levels and estimates the glomerular filtration rate (GFR), a crucial kidney health indicator.

Future enhancements will focus on optimizing real-time interstitial fluid (ISF) measurements and increasing portability, through a mobile app interface. By combining advanced biosensing technology with practical usability, *AptaSense* represents a significant advancement in chronic kidney disease management, making monitoring more accessible and cost-effective for patients.

2. Biosensor

Developing a biosensor involves two crucial mechanisms to detect a desired analyte: the biological recognition element and the transducer, as represented in **Appendix A** (**Figure A1)** (Hall, 1990; Yoo et al., 2020). After these core components have been carefully developed, it is important to design cartridge technology and a user-friendly interface, which are crucial for introducing the biosensor to the market in a way that makes it more accessible, non-invasive, and versatile compared to conventional analytical techniques (Jia et al., 2024; Zhou et al., 2014). In this report, the development of a continuous creatinine monitoring biosensor – *AptaSense* – is described in detail.

To achieve continuous creatinine detection, the **molecular recognition** relied on two novel aptamer-based sensors that were developed for different mediums. Both sensors used anti-creatinine aptamers with the sequence: 5' Thiol-CGACGGTGGCCTATTAAATAGCTTTAGTTTAAGAAAAGTAATAGGGGG TGTCG-Methylene Blue 3' (Ganguly et al., 2024). Initially, surface optimization for creatinine detection was performed in Phosphate-Buffered Saline (PBS) medium. However, the device is unsuitable for ISF due to high stabilization time and low accuracy. Despite this, the PBS results are relevant and are presented in **Appendix B**.

In the case of the detection of creatinine in diluted human serum, a gold Screen-Printed Electrode (SPE) was initially modified by electrodeposition of gold nanoparticles applying a potential of -400 mV for 150 seconds in the presence of a solution of $HauCl_4$ (5 mM) and HCl (0.1 M) (Sharma et al., 2023). These deposited nanoparticles contributed to an improvement in the sensibility of the biosensor. To create a bifunctional surface, able to covalently bind thiol-functionalised aptamers and inhibit non-specific adsorption of interferents, a polydopamine (PDA) and ethanolamine (ETA) film was grown over the previously synthesized nanoparticles. This film was chemically synthesized for 30 minutes using a solution of dopamine (DA) of 5 mM and ETA of 100 mM (Almeida et al., 2021). By depositing a drop of solution of 4 µM of aptamers over the electrode for 24 h, the recognition element was attached to the surface either through a 1,2-Michael addition or a thiol-gold interaction with the gold nanoparticles (Balamurugan et al., 2008; De Girolamo et al., 2018). Finally, to further avoid nonspecific interactions and remove the weakly bound aptamers, a solution of 11-mercapto-1-undecanol of 0.5 mM was deposited for 30 minutes (Balamurugan et al., 2008; De Girolamo et al., 2018). All the referred experimental steps can be interpreted with the help of the scheme depicted in **Figure 1**.

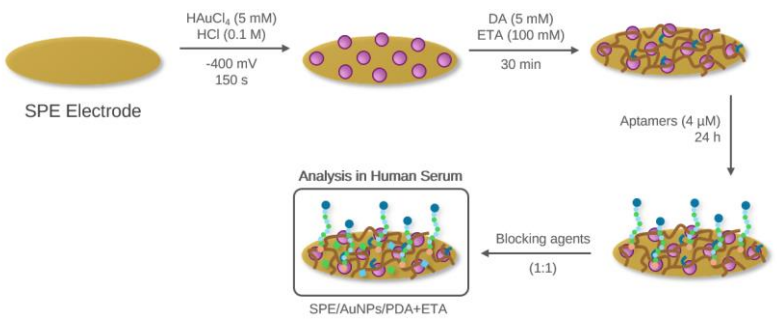


Figure 1 – illustration of step-by-step experimental modification of the SPE electrodes for the detection of creatinine.

Once the aptamers are attached to the surface, the interaction between the recognition element and creatinine needs to trigger a measurable signal, also known as the **physical transduction**. As it is commonly approached in the literature, aptasensors may use the response of a redox probe attached to the 5' end of the aptamer to obtain selective responses (Schoukroun-Barnes et al., 2016). Therefore, the transducer consists of an electrochemical amperometric biosensor, that uses Square Wave Voltammetry (SWV), where the current response increases with the concentration of analyte due to the approximation of the redox probe to the electrode surface. This increase of current is associated with the facilitated charge transfer between the electrode and the redox species, as represented in **Figure A2** (Zhang et al., 2018). The most common redox probes are ferrocene and methylene blue, being the last one selected for the detection of creatinine (Schoukroun-Barnes et al., 2016; Yu et al., 2022).

The **reader instrument** responsible for measuring the current response is a Sensit Smart Potenciostat from Palmsens, which is connected to the SPE. This connection is facilitated by the design of the **microfluidic cartridge**, which was 3D printed in Polylactid Acid (PLA), a material capable of sustaining liquids without severe wear. This cartridge has a drawer to secure the SPE and a cylindrical compartment aligned with the electrode, properly isolated with an O-ring. The sample is inserted into this compartment using a micropipette, and with the help of a peristaltic pump, it flows out to an exit compartment where the return of blood to the body is simulated. This procedure reduces the need of using flushing solutions. The use of this pump will allow for automated sample removal in the future, enhancing the overall functionality and reliability of the biosensor. **Figures 2A** and **2B** show the microfluidic cartridge and an overview of the system.

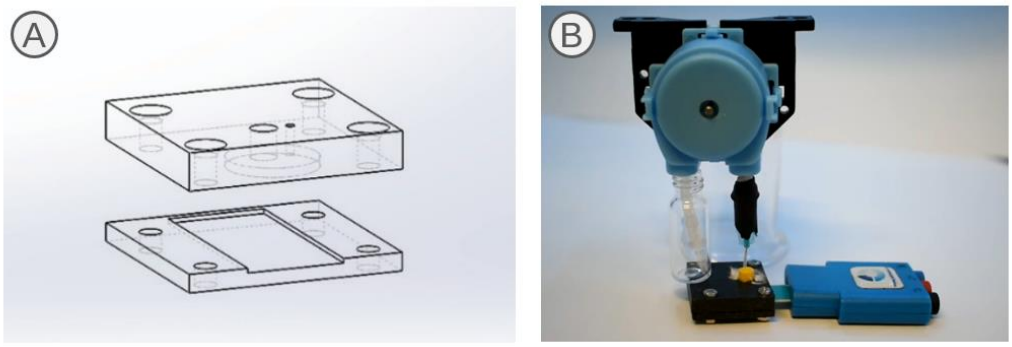


Figure 2 – **A)** Cartridge design and **B)** Overview of the system.

The PSTrace software, used with the potentiostat, enables the acquisition and visualization of SWV data. The current values obtained are processed and analyzed automatically in MATLAB based on a pre-established calibration curve to determine the creatinine concentration accurately. The interface intends to enable **user interaction** in a way that users can visualize their serum creatinine levels and, if desired, input additional information such as sex, age, and race. Based on this input, the software estimates the glomerular filtration rate (GFR) using the equation provided in **Appendix C** (Inker et al., 2012). GFR is considered a more sensitive biomarker for kidney failure than serum creatinine (Lopez-Giacoman & Madero, 2015). Allowing the user to input this information in a simple way provides a more accurate and personalized assessment of kidney health.

3. Technological feasibility

To evaluate the surface modification of the SPE electrode used for the human serum, the response of the film was followed during each step with SWV in the presence of a PBS solution. As shown in **Figure 3A**, the modification of the surface with gold nanoparticles leads to an increase in the current response. Once the polymeric film of PDA and ETA is formed, its poorly conductive character slightly decreases the signal. Next, when the aptamers and the blocking thiol are attached, the current increases again and the signal becomes more significant. Once the polymeric surface is modified with the recognition element, the study through SWV in the presence of a solution of 300 µM of creatinine in PBS shows a significant increase in current, as shown in **Figure 3B**, demonstrating the ability of the aptamers to detect the desired analyte. Additionally, the morphology of the films obtained before the binding of the aptamers was evaluated (on **Appendix D**, **Figure D1**), revealing a less irregular film of PDA with ETA when the gold nanoparticles are deposited underneath the polymer.

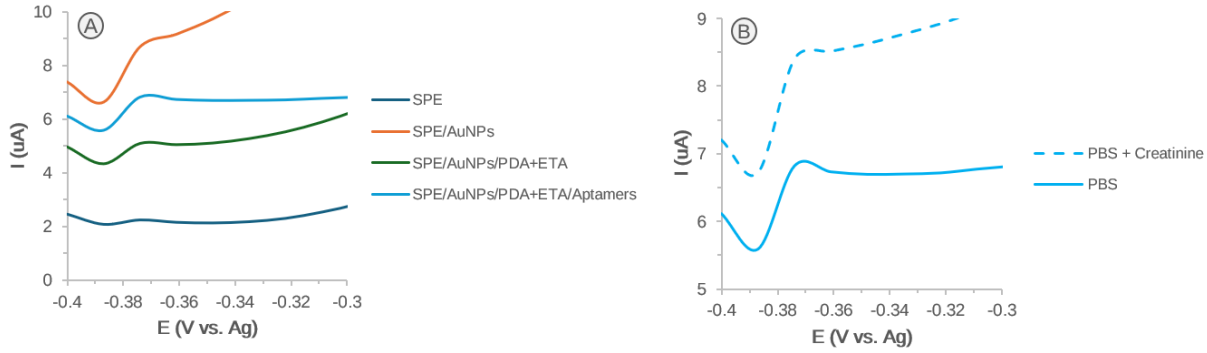


Figure 3 – **A)** SWV curves of modified electrodes in PBS 150 mM (pH= 7.4) and **B)** SWV of the modified electrode with SPE/AuNPs/PDA+ETA/Aptamers in the presence and absence of creatinine in PBS 150 mM (pH=7.4).

Once the surface was successfully modified, the stabilization of the signal response of the electrodes using creatinine in diluted human serum was studied. Therefore, several SWV curves were performed with the same concentration every 2 minutes until the same signal response was obtained. As shown in **Appendix D** on **Figure D2**, in most cases after 4 minutes the signal response was very similar to the ones at 6 and 8 minutes, indicating that the response of the sensor was stabilized around 4 minutes.

Nevertheless, these results showed unexpected responses associated with the decrease of current, as opposed to what was observed when the medium used was PBS. This current decrease might be related with the composition of the human serum, whose larger molecules such as proteins near the electrode surface, might have difficulted the approximation of the redox probe to the surface, and therefore presenting a progressively lower signal.

After this validation, a calibration curve was built considering the current responses obtained at 4 minutes, not considering the outliers, as shown in **Figure 4**.

The analysis of the regression statistics and ANOVA led to an R^2 of 0.9872, a standard error of 0.0839 and an F of 846.63. It can therefore be concluded that since the $R^2 \approx 1$, the standard deviation

is considerably low and that the F is a considerable high value, the linear regression is statistically accurate, and that this calibration curve can be used for the determination of the concentration of creatinine in the diluted human serum. These results prove that, since the diluted human serum simulates the interstitial fluid (ISF) this surface modification together with the recognition element might have a great potential if applied in these real matrixes.

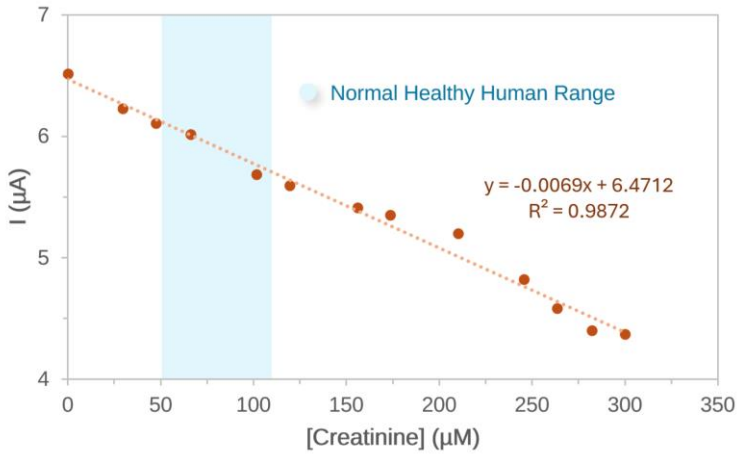


Figure 4 – Calibration curve of the concentration of creatinine in diluted human serum.

Once the calibration curve was constructed, a computational interface was also developed to continuously determine the concentration of creatinine. Considering the results obtained previously and the estimation of the GFR, it was possible to develop a user-friendly interface that determines with accuracy the levels of creatinine. This computational program has been represented in **Figure 5A, B** and **C**.

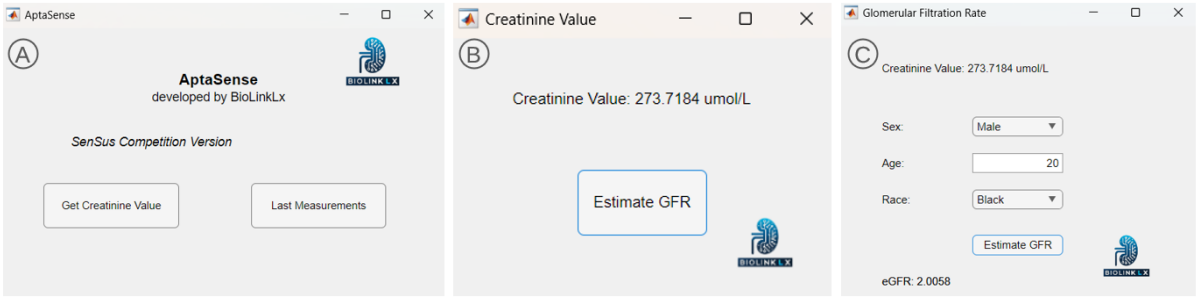


Figure 5 – A) First window of the interface, **B)** second window regarding "Get Creatinine Value" and **C)** third window regarding "Estimate GFR".

It is noticeable that to fully realize the potential of converting this sensor into a wearable device, many challenges need to be overcome. In the case of **molecular recognition**, more studies on reproducibility, selectivity and stability are needed. It is also important to optimize the **microfluidic system cartridge**, potentially incorporating an automated micro-pump and to fully develop a smaller portable device. Finally, the **software** developed for user interaction is currently available only on a computer or laptop, which limits its portability. However, since the potenciostat can be used with a smartphone, there is potential to convert this interface into a mobile app in the future, enabling a more convenient and accessible experience, as shown in **Appendix E** on **Figure E1**.

4. Originality

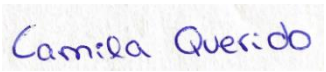
4.1. Team

After reviewing current creatinine detection technologies, we noted that most biosensors are designed for point-of-care rather than continuous monitoring. Our approach uniquely integrates aptamer-based technology with a system for continuous creatinine monitoring. We enhanced sensor sensitivity by depositing a polymeric film of PDA and ETA over electrodeposited gold nanoparticles on the electrode, being a completely novel method for creatinine detection.

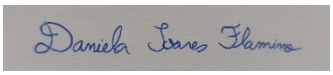
Our microfluidic cartridge is simpler and more cost-effective than existing flow cell attachments. The user-friendly computational interface not only visualizes creatinine levels but also calculates GFR, a clinically relevant CKD index. These innovations make our biosensor a comprehensive tool for continuous kidney function monitoring.

We received valuable support and guidance from our coach and supervisor, allowing us to develop and refine our approach independently.

Team leader,



Sub-Team Leader,

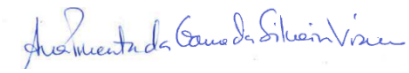


4.2. Team's supervisor

The team from the University of Lisbon has developed an innovative biosensor device for continuous creatinine monitoring. This device combines new approaches with established scientific knowledge, resulting in a sensitive and cost-effective solution compared to existing commercial systems. A key focus was on creating a transducer matrix that efficiently immobilizes the aptamer and transduces a specific signal. Unlike conventional methods that involve multiple complex steps, the team proposed a novel strategy using a hybrid polydopamine-ethanolamine film and a gold nanoparticles layer, which simplifies the process and enhances sensitivity without additional chemical reactions or blocking steps.

Polydopamine, inspired by mussel foot proteins, is biocompatible, adhesive, and allows for robust covalent bonding with proteins, while ethanolamine aids in aptamer distribution and prevents non-specific adsorption. The innovation extends beyond the sensing interface to the overall design of the electrochemical device, which includes a simple method for blood collection and continuous measurement via a microfluidic system.

I was extremely impressed with the team's performance and commitment throughout our meetings and the extensive lab assays to validate the transducer interface. Their focus on creating a versatile, user-friendly, and low-cost device drove every discussion with scientists and urologists. The final document reflects the quality of their work and the rigorous evaluation of proposal potential and associated risks.

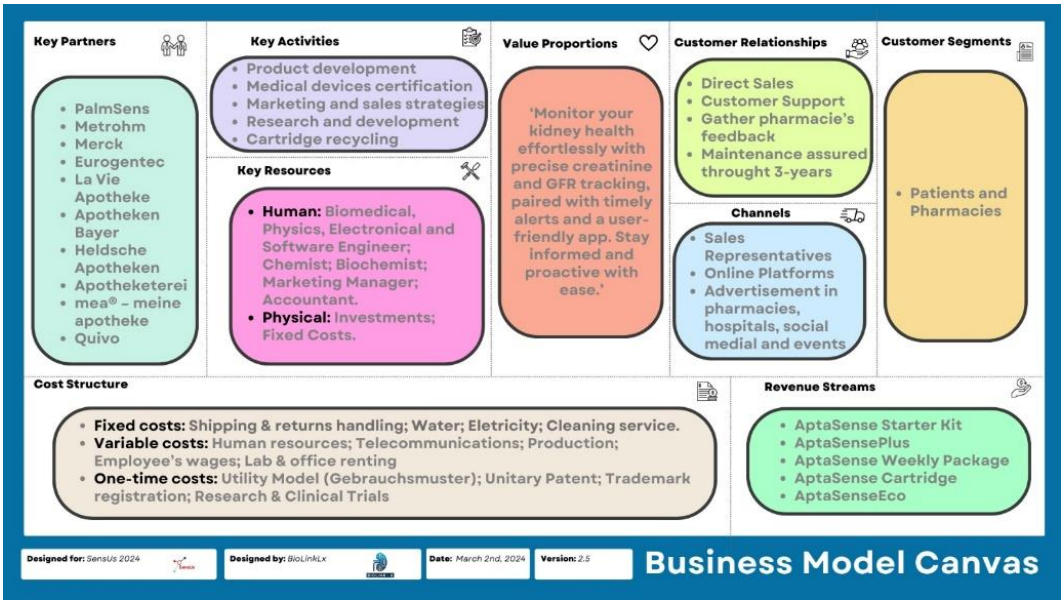


Associate Professor

Chemistry and Biochemistry Department; Faculty of Sciences, University of Lisbon

5. Translation Potential

5.1. Business Model Canvas



5.2. Stakeholder Desirability

Approximately 850 million people have kidney disease, representing around 10% of the worldwide population (Francis et al., 2024). Chronic kidney disease (CKD) is when one or both kidneys lose their renal function for at least 3 months since proper medical observation (*Facts About Chronic Kidney Disease*, 2020). Since it is a non-communicable disease characterized by a very slow evolution and an asymptotic behavior, it is divided into 5 stages, as shown in **Appendix F** on **Figure F1** (*Stages of Chronic Kidney Disease (CKD)*, 2023). According to Kovesdy (2022), 10.6% of CKD patients are in stages 3-5, with significant symptoms appearing only in stages 3b/4, such as problems in urinating, itchy and/or dry skin, nausea, trouble concentrating, vomiting, shortness of breath and cramping (*Facts About Chronic Kidney Disease*, 2020; Kovesdy, 2022). Early diagnosis and continuous monitoring of this disease are crucial to prevent significant progression, which is the primary goal of *AptaSense*.

Regarding a B2C approach, *AptaSense* targets: individuals with CKD, which can progress rapidly to kidney failure (*Kidney Failure*, 2024); kidney transplant patients in need to monitor for allograft loss or rejection - after the first year, they see their nephrologists less often and have labs drawn only every few months (*Limitations of Relying on Creatinine After Kidney Transplant*, n.d.); and people who have some of the risk factors of CKD, such as diabetes, hypertension, heart disease, obesity, old age, family history of CKD or kidney failure, personal history of acute kidney injury, smoking, autoimmune conditions, amongst others (*Facts About Chronic Kidney Disease*, 2020). It is also important to consider a B2B2C approach, highlighting the importance of pharmacies as the main distribution channel due to their wide consumer reach.

To determine the essential features for *AptaSense* to meet stakeholder needs, a validation process is crucial. In an online meeting on April 23rd, Dr. Rui Manuel Batista Alves, head of the Nephrology Department at Coimbra Hospital and University Center, stated that creatinine is not the most

appropriate biomarker for kidney failure due to influences like muscle mass. However, serum creatinine measurement enables the determination of GFR, the most clinically relevant index of kidney function. A biosensor for continuous creatinine monitoring benefits patients and healthcare professionals by tracking biomarker variations between appointments. A transcript of the interview is available in Appendix G.

Based on this clinical necessity and posts written by patients on forums such as “Chronic Kidney Disease Forum”, *AptaSense* aims to meet the needs of patients and healthcare professionals. It will aid in monitoring kidney health, preventing unnoticed disease progression, and reducing healthcare costs by minimizing hospital visits. The device will provide timely alerts on creatinine levels and GFR fluctuations, enhancing patient empowerment, health literacy, condition management, and reducing disease-related stress.

To sum up, a profitable customer would be someone who fits our target segment, has insurance coverage for continuous monitoring devices and/or needs a creatinine monitoring device.

For stakeholders, **patients** need reliable, easy-to-use and accurate monitoring of their kidney health. **Doctors** require real-time data and a database to make more informed clinical decisions about disease progression since the last appointment. **Hospital management** seeks cost-effective solutions, such as *AptaSense*, that enhance patient care and reduce hospital readmissions. **Insurance companies** are interested in devices that lower overall healthcare costs by preventing severe complications, and may offer the biosensor as a covered benefit, thereby reducing long-term treatment costs.

According to Rule 10 of the Medical Device Coordination Group (MDCG 2021-24), *AptaSense* is classified as a class IIb medical device.(*MDCG 2021-24 - Guidance on Classification of Medical Devices - European Commission*, n.d.) As specified by Article 5 of Chapter 2 of the Medical Device Regulation (MDR), devices must fulfill general performance and safety requirements to be marketed in Europe (“Article 5 Archives,” n.d.). The CE mark, required for commercialization in the EU, ensures compliance with these regulations and indicates that the device has been assessed by a notified body (*Medical Devices (MD) - FAQ*, n.d.). As we plan to focus on the German market in the first five years, obtaining the CE mark is essential.

Given *AptaSense*'s access to patient data, ensuring compliance with the General Data Protection Regulation (GDPR) is essential. GDPR, a globally influential law, unifies EU directives on personal data handling and applies to all smartphone apps processing EU citizens' data, irrespective of the app's location or purpose. The GDPR's core principles—consent, privacy, security, and fair data collection—offer clear guidance on properly managing smartphone-acquired personal data (Ross et al., 2023; *What Is GDPR, the EU’s New Data Protection Law?*, 2018).

The competitor analysis (**Appendix H**) highlights the *AptaSense*'s main advantage: continuous creatinine monitoring, alongside with all the characteristics mentioned previously. To ascertain the innovative nature of the developed biosensor, a search for patents was carried out on Google Patents and ESPACENET. The most relevant ones are summarized in **Appendix I**. According to the intellectual property analyzed, there isn’t any patent for an aptamer-based amperometric continuous creatinine

monitoring biosensor like *AptaSense*, which ensures the innovation necessary to guarantee the patentability of the product.

5.3. Business feasibility

5.3.1. Key Resources

As mentioned in **Appendix J**, in 2025 and 2026 we will continue to invest in research and clinical trials of our biosensor since *AptaSense*, in terms of Technology Readiness Level (TRL), is in the beginning on level 4 and needs improvement to reach the final prototype, ready to be commercialized. In terms of research, it is necessary to rent a functional laboratory equipped with high-quality materials, to optimize the molecular recognition and transduction of the biosensor, especially to reduce production costs. The cartridge's design will be improved by continuous microfluidic studies and based on patients' and healthcare professionals' feedback.

As a tool of clinical trials' quality assessment, it is crucial to continuously obtain feedback from users and healthcare professionals to understand the main features they value in *AptaSense*. These features should be highlighted by the marketing team when the product finally enters the market.

It is also fundamental to hire a team of STEM specialists, described in the Human Key Resources on the Business Model Canvas, to assist in the company's mass biosensor production.

5.3.2. Key Activities

Our marketing and sales strategy will involve direct sales to pharmacies and our website, digital marketing via social media and health sites, and participation in kidney healthcare conferences. This approach targets potential clients, doctors, and researchers for clinical trials and endorsements.

Key activities include R&D, quality assurance, regulatory compliance, and forming partnerships with German distributors.

To protect intellectual property, we will first apply for a utility model (*Gebrauchsmuster*) in Germany for clinical trials and publications. In 2026, we will seek a unitary patent for EU-wide protection, facilitating market expansion post-2032.

5.3.3. Key Partners

As we launch our startup in Germany, it is crucial to establish strategic partnerships with hospitals specializing in nephrology, so they can recommend and integrate our product into the daily care of patients, thus increasing the credibility and reach of the device. Our key partners include PalmSens, Metrohm, Merck and Eurogentec which provide essential solutions, reagents, biological components, and technical support for the development of our biosensors. Additionally, we are looking to establish partnerships with the largest pharmacy chains in Germany, such as Rossmann, dm-drogerie markt, Sanicare, and mea® - meine apotheke, to ensure wide and efficient distribution of our products. These collaborations are fundamental to ensuring the continuous supply of high-quality materials and integrating our product into the daily care of patients.

5.3.4. Sustainability

In our project sustainability is a core principle reflected in our *AptaSenseEco*, a discount on the cartridge system customers get access to when they return their used cartridges. In this way, we are assisting in environmental waste reduction by recycling cartridges' material to produce new ones. The biosensor's cartridge is 3D printed using PLA, a sustainable polymer derived from renewable resources. PLA biodegrades into carbon dioxide and water, with a biodegradation rate of about 1600 kg per metric ton, making it more environmentally friendly than alternatives like polyethylene terephthalate (PET), which degrades at 4150 kg per metric ton (Trivedi et al., 2023). Through efficient waste management and rigorous recycling efforts, we are committed to maintaining sustainability in all aspects of our operations, ultimately aiming to lessen our carbon footprint and promote a greener future.

5.4. Financial viability

5.4.1. Cost projection

The current manufacturing cost of *AptaSense* is divided into 3 components, as illustrated in **Figures K1** to **K3** (**Appendix K**): biosensor, SPE and medical tape. The biosensor, including the cartridge system and the reader instrument, costs €499.87 per unit, considering the special 50% discount PalmSens provided for the potentiostat in this competition. The SPE includes the electrode and all the reagents for surface modification and molecular recognition, and costs €17.99 per unit. The medical tape serves to assist in skin fixation, and costs €0.03 per unit. In total, *AptaSense* production cost is €517.89 per unit, only in materials. It is expected that these costs can be lowered through research and by optimizing the prototype.

Fixed costs include water, electricity, and outsourced cleaning services. Variable costs cover laboratory and office rent (increasing 4% annually), production materials, telecommunications, wages (subject to inflation and biennial increases), HR outsourcing (2% of gross salaries), and shipping and returns outsourcing (€499 monthly plus €0.17 per shipment). Investments include utility model submission, patent, CE certification, trademark registration, and funding for research and clinical trials. Further details are in **Figures K5** to **K10**.

5.4.2. Sales price

AptaSense offers five main products:

- ***AptaSense Starter Kit***: Includes one biosensor, one SPE, and 14 medical tapes. Priced at €549.99 with a production cost of €518.30.
- ***AptaSense Weekly Package***: Includes one SPE and 14 medical tapes for one week of usage. Priced at €22.49 with a production cost of €18.43. The monthly price is €89.99 with a production cost of €73.72.
- ***AptaSensePlus***: Our monthly subscription model, available on our website, offers a discount on four AptaSense Weekly Packages. Priced at €77.49 per month with a production cost of €73.72, it includes weekly automatic shipments.
- ***AptaSense Cartridge***: Includes one biosensor (without reader instrument or pump), one SPE, and 14 medical tapes. Priced at €39.99 with a production cost of €20.00.

- *AptaSenseEco*: Discount for returning old cartridges to the company for recycling. Priced at €29.99 with a production cost of €20.00.

Given Germany's CKD prevalence and 18,000 pharmacies, the assumption is that 125,000 customers will buy the Starter Kit in the first year, totaling 24,950 sales across all product options (*Density of Pharmacies in the EU | ABDA*, n.d.). Sales projections and cumulative annual growth for each product are detailed in **Figures K11** and **K12**.

5.4.3. Market analysis

In 2022, the Creatinine Test Market was valued at $0.9 billion and is projected to reach $3.36 billion by 2032, reflecting a CAGR of 15.80% (Gotadki, 2019). Europe, particularly Germany, is a key market due to high CKD prevalence (10-13% among German adults) and advanced healthcare infrastructure (Ärzteblatt, n.d.; Gotadki, 2019). *AptaSense*'s initial target is the German market, which is large enough to justify development costs and offers significant revenue potential due to high CKD rates.

According to the competitor analysis in the **Appendix H**, current monitoring options include blood tests (minimally invasive but non-continuous), urine tests (simple but require regular sample collection), and imaging tests (non-invasive but costly and impractical for frequent use). Existing home devices like the *StatSensor Xpress Creatinine* by Nova Biomedical are not continuous, unlike our biosensor, which will continuously monitor creatinine levels in interstitial fluid (*StatSensor® and StatSensor Xpress® Creatinine and eGFR Meters*, n.d.).

Market penetration for new devices might be slow initially, but the favorable adoption of continuous glucose monitoring (CGM) devices suggests optimism. Reimbursement for CGM in Germany since 2016 and intermittent scanning CGM since 2019 supports the feasibility of reimbursement for *AptaSense* (Auzanneau et al., 2021).

Looking beyond Germany, significant growth is anticipated in other markets. The UK shows rapid growth in Europe, while Asia is expected to have the highest CAGR, with India growing the fastest and China holding the largest market share. The US leads globally with substantial growth prospects due to advanced technologies and major industry players (Gotadki, 2019).

5.4.4. Revenue streams and business strategy

Important data on total costs, revenues, and the financial analysis spreadsheet are present in **Figure K13** (**Appendix K**). Revenue primarily comes from biosensor sales and subscriptions, with net sales rising from €18,057,041 in 2028 to €25,275,845 in 2032. **Figure K14** shows the cumulative net project cash flows over time. The payback period is about 3 years and 7 months, reaching break-even point at around 19,023 sales or €13,767,291.18, visible in **Figure K15**.

In terms of business intelligence, systems to collect data from biosensors, including patient health metrics and device performance while ensuring GDPR, are essential. Advanced analytics software provides real-time monitoring and predictive analytics. With continuous user feedback from the app and/or official website, the company can optimize operations, enhance customer satisfaction, and drive revenue growth.

6. Team and support

BioLinkLx's team members are:

- **Beatriz Santos**: studying Biomedical Engineering, she was part of the Business Team. She helped creating content for social media and she also organised meetings with other teams.
- **Camila Querido:** studying Biomedical Engineering, she was the Team Leader, responsible for communicating with the SensUs organization and partners. She contributed mainly to the development of the cartridge and the computational interface.
- **Daniel Carvalho:** studying Chemistry, his work was mostly focused on the electrode surface modification and molecular recognition.
- **Daniela Flamino:** studying Biomedical Engineering, she was the Sub-Team Leader, responsible for the Business Team and for keeping track of deadlines. She contributed to the molecular recognition work and gathered important feedback from professionals.
- **Filipa Branco:** studying Biomedical Engineering, she was part of the Business Team, helped with the improvement of the cartridge and was also active in social media.
- **Francisco Duarte:** studying Chemistry, he contributed to the surface modification work and played a role in managing external relations.
- **Guilherme Simões:** studying Biomedical Engineering, he played a role in the development of the cartridge and software tasks, like the computational interface.
- **Katherine Bettencourt:** studying Chemistry, she was essential in developing the aptamer-based biosensor, including all the steps of the surface modification and physical transduction. She also played a crucial role in bridging the different aspects of the project.
- **Luísa Maria:** studying Biomedical Engineering, she was present in meetings with partners and gathered valuable insights for the project.
- **Sara Félix:** studying Biomedical Engineering with a Biochemistry background, she contributed to the electrode's surface modification and was present in important meetings with partners.

We extend our deep appreciation to **Beatriz Sequeira-Antunes**, our Coach, for her valuable insights into aptamer-based molecular recognition and cartridge technology. We also express our gratitude to **Prof. Ana Viana**, our supervisor, for her guidance on molecular recognition, **Prof. António Cascalheira** and **Prof. Jorge Correia** for their support in developing the microfluidic cartridge, and **Prof. María Castañón** for her advice on the use of aptamers. Additionally, we thank **Prof. Teresa Vieira** for her assistance with our business model and acknowledge the financial support from **FCiências.ID**, which enabled us to acquire essential materials. Our thanks go to **PalmSens** and **Metrohm (Pomensoro)** for their valuable partnership and for providing us with the necessary equipment. Finally, we are grateful to our ex-team members, **Carolina Fernandes**, **Carolina Abreu**, **Andreia Araújo**, **Paula Menezes**, and **Daniel Bento**, for their contributions to marketing activities and initial business model development.

7. Final Remarks

AptaSense is an innovative device that enables continuous and accurate measurement of creatinine levels, and currently no other products on the market utilize the same principle. Looking ahead, we plan to optimize the system to ensure its applicability in real ISF and enhance its portability.

The diagnosis and management of kidney failure typically involve blood and urine analysis performed by medical professionals, requiring frequent hospital visits (Chen et al., 2019). The development of this biosensor represents a significative advancement in continuous creatinine detection, making the management of this disease more accessible and cost-effective for patients.

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9. Appendix

Appendix A – Biosensors and Detection Mechanisms

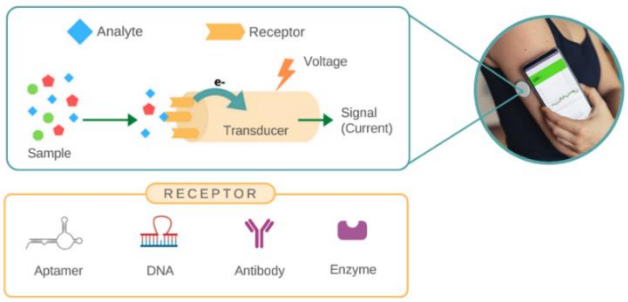

Figure A1 – General scheme of a biosensor. Adapted from the literature (Martynko & Kirsanov, 2020; Sande et al., 2021).

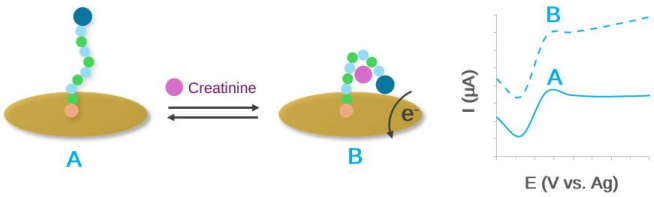

Figure A2 – Mechanism of transduction and reversibility of aptamers.

Appendix B – Surface Modification for the Analysis of Creatinine in PBS

As referred to in section 2, a novel surface for the detection of creatinine in PBS was successfully developed. Although these results cannot be considered for its use in the ISF, we considered this as a relevant scientific development. For the detection of creatinine in PBS medium, the SPE electrode was also modified with gold nanoparticles and then PDA with ETA. To increase the response of the sensor, another layer of gold nanoparticles was electrodeposited over the polymeric surface with a solution of $HauCl_4$ (5 mM) and HCl (0.1 M) and applying a potential of -400 mV, now for 75 seconds. After this step the deposition of the aptamer and blocking agent was performed in the same manner as referred to in section 2. **Figure B1** represents the Atomic Force Microscopy (AFM) image of the surface modification before the attachment of the aptamers, where it is clearly possible to observe the presence of nanoparticles of gold on the polymer surface.

After the modification with the recognition element, a calibration curve (**Figure B2**) was constructed and statistically evaluated (**Table B1**, **B2** and **B3**). As in the case with the diluted human serum, the analysis of the regression statistics and ANOVA led to an R^2 of 0.95817, a standard error of 0.2216 and an F of 206.14. It can therefore be concluded that since the $R^2 \approx 1$, the standard deviation is considerably low and that the F is a considerable high value, the linear regression is statistically accurate, and that this calibration curve can be used for the determination of the concentration of creatinine in PBS.

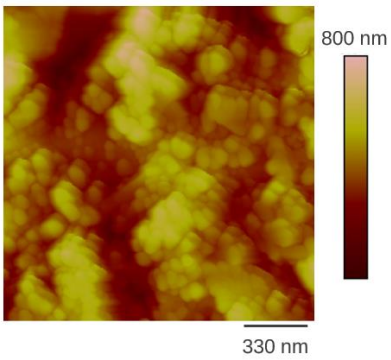

Figure B1 – Modified electrode surface with SPE/AuNPs/PDA+ETA/AuNPs.

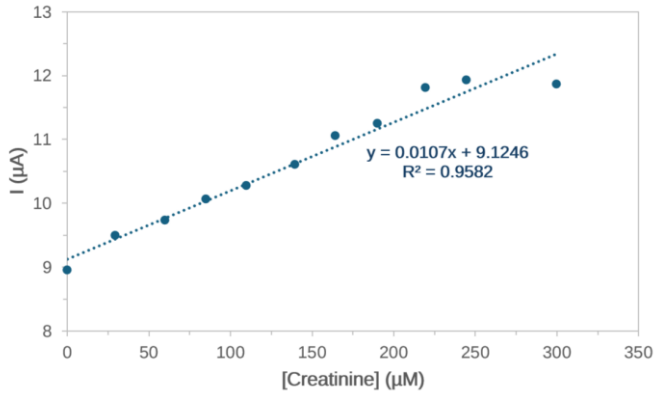

Figure B2 – Calibration curve of the concentration of creatinine in PBS.

Table B1 – Regression statistics results of the calibration curve for the PBS.

Regression Statistics	
Multiple R	0.9789
R Square	0.9582
Adjusted R Square	0.9535
Standard Error	0.2216
Observations	11

Table B2 – ANOVA analysis results of the calibration curve for the PBS.

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	10.1249	10.1249	206.1378	1.65E-07
Residual	9	0.4421	0.0491		
Total	10	10.5670			

Table B3 – ANOVA analysis results of the samples of the calibration curve for the PBS.

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	9.1246	0.1243	73.3984	8.18E-14	8.8434	9.4059	8.8434	9.4059
X Variable 1	0.0107	0.0007	14.3575	1.65E-07	0.0090	0.0124	0.0090	0.0124

Appendix C – Equation for estimating GFR

Equation C1:

$$GFR = 141 \times \left[\min\left(\frac{SCr}{k}, 1\right)^{\alpha}\right] \times \left[\max\left(\frac{SCr}{k}, 1\right)^{-1.209}\right] \times 0.993^{Age} \times s \times r$$

where:

- SCr stands for Serum Creatinine
- $k = 0.7$ for females and $k = 0.9$ for males
- $\alpha = -0.329$ for females and $\alpha = -0.411$ for males
- $s = 0.742$ for females and $s = 1$ for males
- $r = 1.212$ for Black People and $r = 1$ for other races.

Appendix D – Stabilization and Statistical Studies for the Calibration Curve in Human Serum

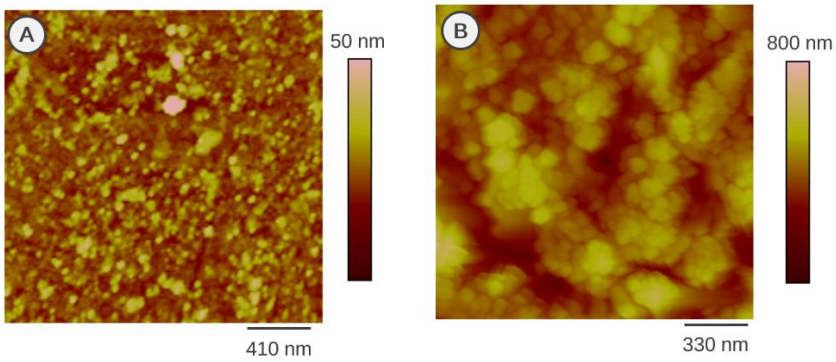

Figure D1 – A) AFM image of PDA+ETA and **B)** AFM image of the modified electrode SPE/AuNPs/PDA+ETA.

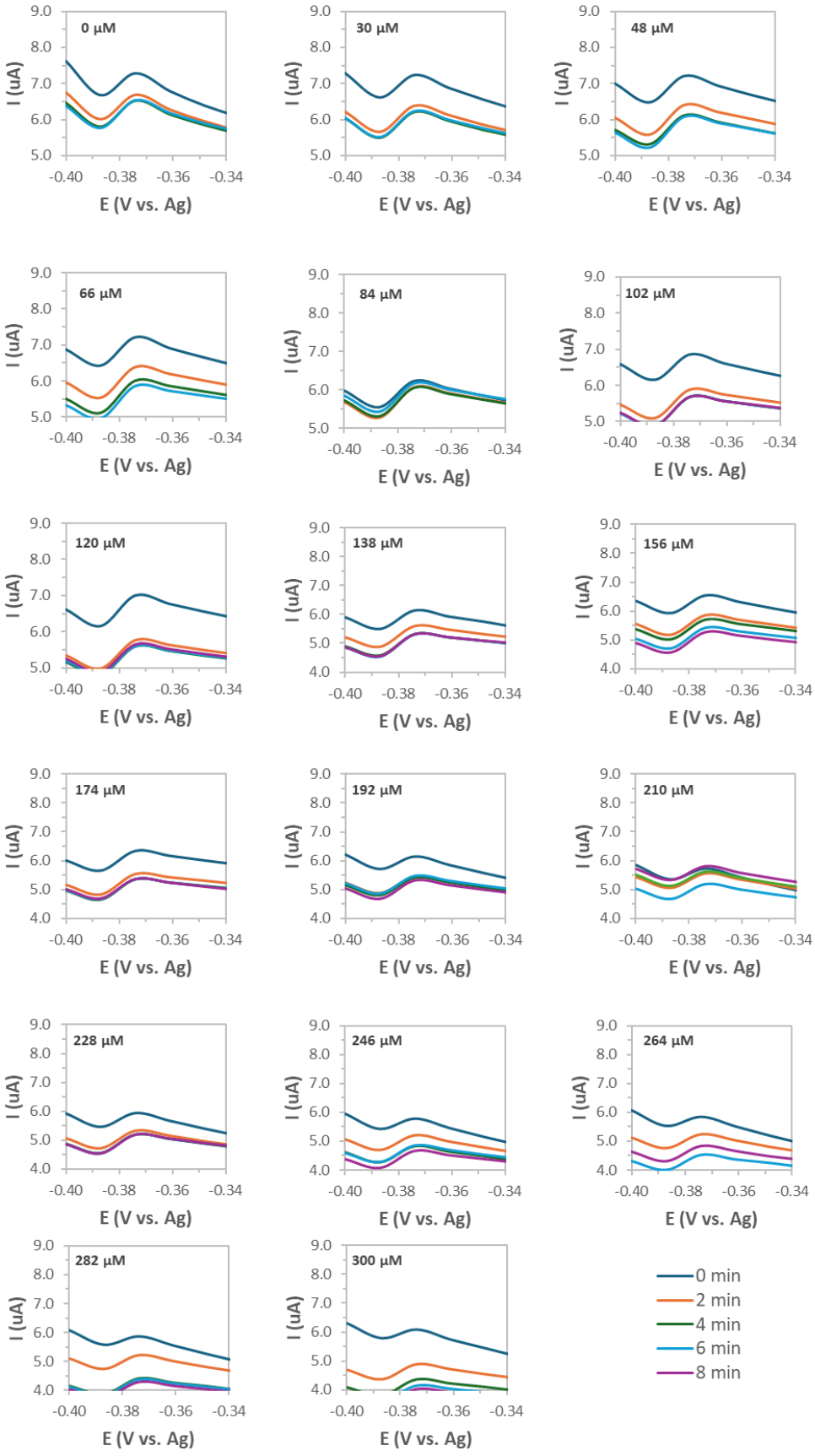

Figure D2 – SWV stabilization study of the modified surface after contact with different concentrations of human serum.

Table D1 – Regression statistics results of the calibration curve for the diluted human serum.

Regression Statistics	
Multiple R	0.9936
R Square	0.9872
Adjusted R Square	0.9860
Standard Error	0.0840
Observations	13

Table D2 – ANOVA analysis results of the calibration curve for the diluted human serum.

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	5.9680	5.9680	846.6377	9.2949E-12
Residual	11	0.0775	0.0070		
Total	12	6.0455			

Table D3 – ANOVA analysis results of the samples of the calibration curve for the diluted human serum.

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	6.4712	0.0435	148.9344	1.5668E-19	6.3756	6.5669	6.3756	6.5669
X Variable 1	-0.0069	0.0002	-29.0970	9.2949E-12	-0.0075	-0.0064	-0.0075	-0.0064

Appendix E – Future Interface Design

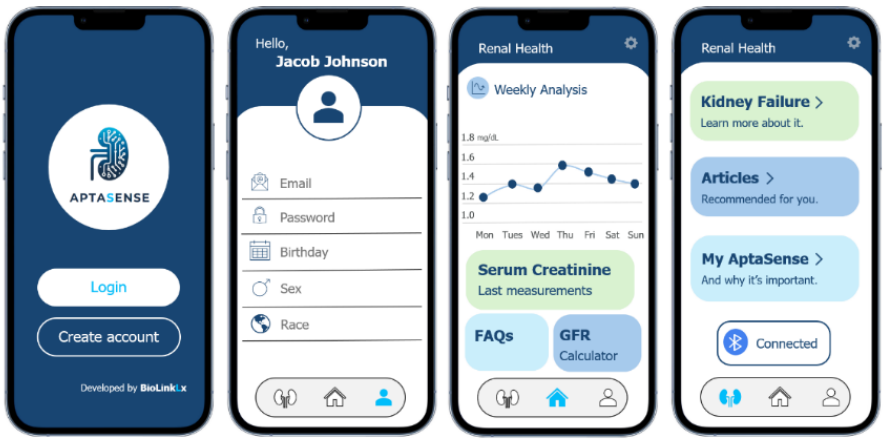

Figure E1 – Design of a future *AptaSense* app.

Appendix F – Stages of Chronic Kidney Disease (CKD)

Figure F1 – Stages of Chronic Kidney Disease. [3]

Appendix G – Online Meeting with Healthcare Professional

(April 23rd, 3:33 pm. The following meeting was in Portuguese.)

Daniela Flamino (BioLinkLx): Thank you for allowing the recording of this meeting. To start, what are the main obstacles that people diagnosed with chronic kidney disease face nowadays?

Dr. Rui Alves: Chronic kidney disease progresses through five stages, from 1 to 5, with stage 3 further divided into 3a and 3b. The higher the stage, the more incapacities the patient will experience. A person can be asymptomatic from stages 1 to 3. From stage 3b/4 onward, the disease can severely affect the patient's lifestyle and common activities, such as walking.

Daniela Flamino (BioLinkLx): How often does a patient with chronic kidney disease need to visit the hospital to monitor the progression of the disease?

Dr. Rui Alves: It depends on how quickly the disease progresses, which can vary greatly from patient to patient. Older patients usually experience a slower rate of progression. Generally, patients have around 2-3 annual doctor's appointments, but more severe cases may require visits every 2-3 months. The frequency depends on risk factors such as age and comorbidities like hypertension.

Daniela Flamino (BioLinkLx): In your opinion, is creatinine the best biomarker for kidney disease?

Dr. Rui Alves: The glomerular filtration rate (GFR) is clinically more relevant than serum creatinine, as it is a more reliable measure of renal function. Serum creatinine levels can be influenced by factors such as muscle mass. In kidney disease, nephron mass often decreases, but creatinine levels might appear normal because the remaining nephrons adapt, which can be misleading. However, serum creatinine is used to calculate GFR, so while creatinine alone is not the best biomarker, it is still part of the GFR calculation.

Daniela Flamino (BioLinkLx): Is there a need for a medical device, such as a biosensor, that continuously monitors kidney health by measuring serum creatinine and GFR values from interstitial fluid samples?

Dr. Rui Alves: This is a very interesting idea, especially for those with kidney failure. One of the most common questions I get from my patients is about their latest creatinine levels. Continuous home monitoring could be highly valuable, necessary, and productive. It is important for patients to learn how to use such a device and recognize significant fluctuations in creatinine levels so they can schedule doctor's appointments sooner if needed. I wish you good luck and look forward to hearing the results of the competition!

Appendix H – Competitor Analysis

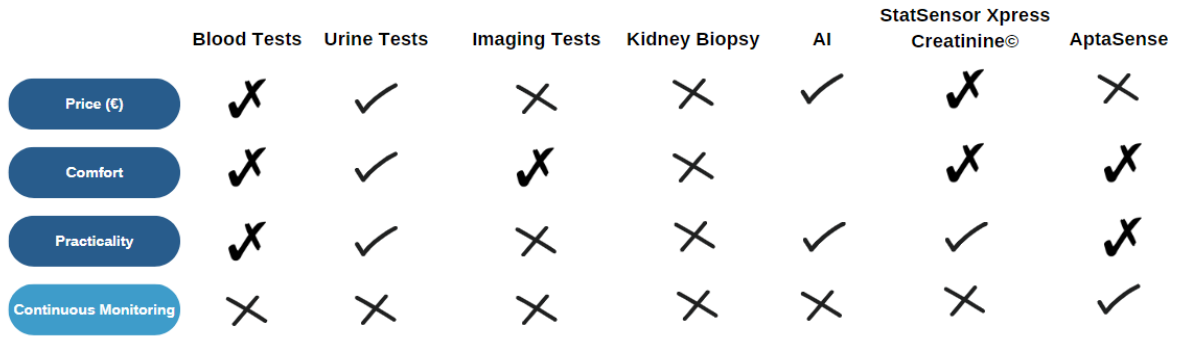

Figure H1 – Competitor Analysis.

Appendix I – Patents

Patent nº 1

Name of Patent: Biosensor for dialysis therapy

Patent Number: US20030216677A1

Inventors: Li Pan, Ramesh Wariar, Leo Martis, Cliff Holmes, Robert Childers, Shahid Din

Short summary: This invention is an enzymatic biosensor for monitoring the total solutes removed from a patient during dialysis therapy, capable of measuring creatinine levels as well as other biomarkers. The patent claims do not specify that the biosensor is designed for continuous monitoring.

Patent nº 2

Name of Patent: Amperometric creatinine biosensor with immobilized enzyme-polymer composition and systems using same, and methods

Patent Number: US20170260560A1

Inventors: Stephen A . Merchant

Short summary: This invention is an amperometric creatinine biosensor. The patent claims indicate that the biosensor can be used for continuous monitoring, but it does not mention the use of aptamers in its design; instead, it employs enzymes immobilized on an electrode surface.

Patent nº 3

Name of Patent: Device and method for detecting creatinine and albumin to creatinine ratio

Patent Number: WO2016181229A1

Inventors: Vinay Kumar, Navakant Bhat, Nikhila Kashyap, Dhanvantari Madhuresh

Short summary: This invention is an electrochemical biosensor for measuring the creatinine and albumin-to-creatinine ratio. The patent claims do not specify that the biosensor is designed for continuous monitoring.

Appendix J – Time to market

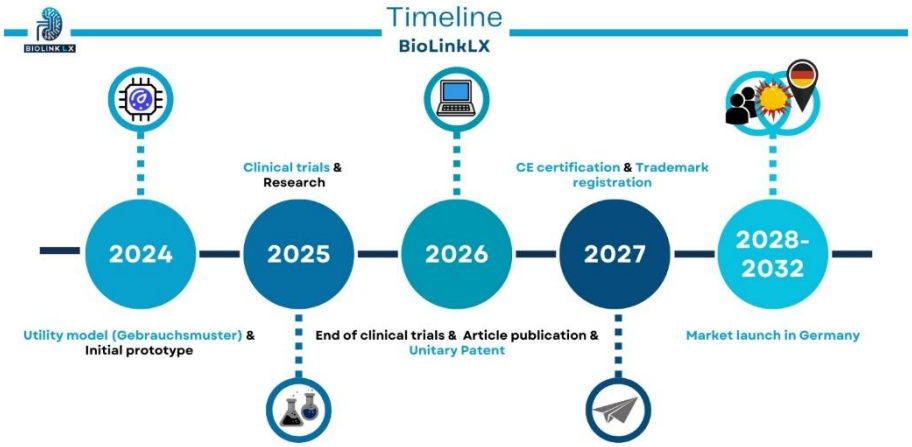

Figure J1 – Time to market.

Appendix K – Financial viability

Materials	Quantity	Cost per Unit	Total Cost
PLA filament	1	15.46€/kg	0.05 €
Zinc-plated steel screw	4	0.01 €	0.05 €
Stainless steel hexagon nut	4	0.01 €	0.05 €
Peristaltic pump	1	4.27 €	4.27 €
Syringe tip	1	1.02 €	1.02 €
Sensit Smart	1	494.03 €	494.03 €
Silicone Tubing	1	0.12 €	0.12 €
3ml Sample Glass Vials	1	0.29 €	0.29 €
Subtotal	499.87 €		

Figure K1 – Cost of biosensor's materials.

Materials	Total Cost
SPE	4.79 €
Gold(III) chloride trihydrate	2.55 €
Sulfuric acid concentrate	0.004 €
Dopamine	0.00005 €
Etanolamine	0.01 €
Aptamer	10.63 €
Subtotal	17.99 €

Figure K2 – Cost of SPE's materials.

Materials	Cost per Unit	Total Cost
Medical Tape	26.56 €	0.03 €

Figure K3 – Cost of medical tape's materials.

	2024	2025	2026	2027	2028
Shipping & Returns handling (outsourcing)/year	- €	- €	- €	- €	10,229.50 €
Human Resources (outsourcing)/month	- €	- €	- €	- €	455.10 €
Telecommunications/month	- €	- €	- €	- €	109.54 €
Production/year	- €	- €	- €	- €	16,238,639.96 €
Employee's wages/year	- €	- €	- €	- €	194,446.30 €
Lab + Office rental/month	- €	- €	- €	- €	3,231.27 €
Total/year	- €	- €	- €	- €	16,488,866.69 €

Figure K4 – Variable costs from 2024 to 2028.

2029	2030	2031	2032
11,077.80 €	10,752.59 €	11,127.95 €	11,312.74 €
499.96 €	515.66 €	519.36 €	535.69 €
109.54 €	119.49 €	119.49 €	129.44 €
19,486,367.95 €	20,297,574.89 €	21,920,713.84 €	22,731,920.78 €
427,642.50 €	476,690.14 €	480,142.90 €	527,205.70 €
3,360.52 €	3,494.94 €	3,634.74 €	3,780.13 €
19,972,728.55 €	20,834,578.69 €	22,463,267.76 €	23,323,782.34 €

Figure K5 – Variable costs from 2029 to 2032.

	2024	2025	2026	2027	2028
Water/month	- €	- €	- €	- €	24.88 €
Electricity/month	- €	- €	- €	- €	135.00 €
Cleaning service/month	- €	- €	- €	- €	837.00 €
Total/year	- €	- €	- €	- €	6,940.56 €

Figure K6 – Fixed costs from 2024 to 2028.

2029	2030	2031	2032
24.88 €	24.88 €	24.88 €	24.88 €
135.00 €	135.00 €	135.00 €	135.00 €
837.00 €	837.00 €	837.00 €	837.00 €
11,962.56 €	11,962.56 €	11,962.56 €	11,962.56 €

Figure K7 – Fixed costs from 2029 to 2032.

Utility Model (Gebrauchsmuster)	490.00 €
Unitary Patent	4,400.00 €
CE certification	5,000.00 €
Trademark registration	650.00 €
Research + Clinical Trials	5,000,000.00 €
Total	5,010,540.00 €

Figure K8 – Investments.

Total Costs				
2024	2025	2026	2027	2028
490.00 €	2,500,000.00 €	2,504,400.00 €	5,650.00 €	16,495,807.25 €

Figure K9 – Total costs from 2024 to 2028.

2029	2030	2031	2032
19,984,691.11 €	20,846,541.25 €	22,475,230.32 €	23,335,744.90 €

Figure K10 – Total costs from 2029 to 2032.

Production Costs: financial assumptions						
Quantity of AptaSense Starter Kit sold at the beginning of commercialization:	12500					
Quantity of AptaSensePlus sold at the beginning of commercialization:	5000					
Quantity of AptaSense Weekly Package sold at the beginning of commercialization:	6000					
Quantity of AptaSense Cartridge sold at the beginning of commercialization:	1000					
Quantity of AptaSenseEco sold at the beginning of commercialization:	450					
Total	24950					
(Regarding the first year of sales)		**2028**	**2029**	**2030**	**2031**	**2032**
Cumulated Annual Sale Growth of AptaSense Starter Kit		15%	20%	25.0%	35.0%	40.0%
Cumulated Annual Sale Growth of AptaSensePlus		15%	20%	25.0%	35.0%	40.0%
Cumulated Annual Sale Growth of AptaSense Weekly Package		15%	20%	25.0%	35.0%	40.0%
Cumulated Annual Sale Growth of AptaSense Cartridge		15%	20%	22.5%	30.0%	32.5%
Cumulated Annual Sale Growth of AptaSenseEco		15%	20%	22.5%	30.0%	32.5%

Figure K11 – Production Costs: financial assumptions.

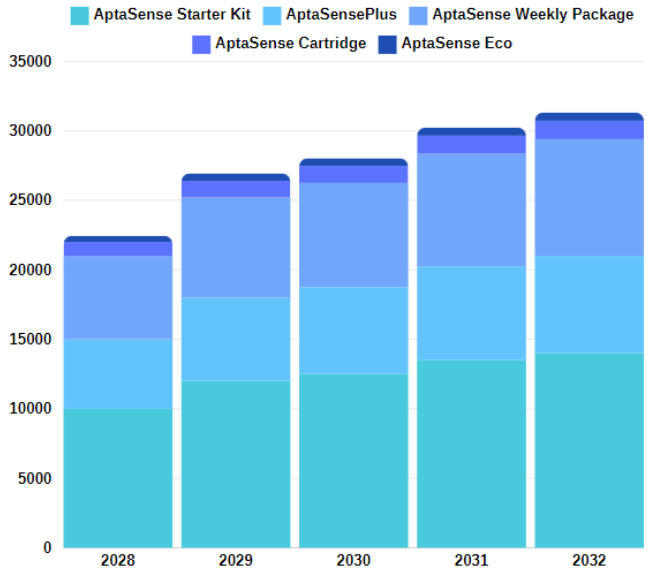

Figure K12 –Number of annual sales of each production option.

(Values in €)		Investment				Operating Phase				
	Assumptions	**2024**	**2025**	**2026**	**2027**	**2028**	**2029**	**2030**	**2031**	**2032**
Net Sales						18,057,041	21,668,449	22,569,963	24,374,330	25,275,845
Variable Charges						16,488,867	19,972,729	20,834,579	22,463,268	23,323,782
Contribution Margin						1,568,174	1,695,720	1,735,385	1,911,063	1,952,063
Cash Fixed Charges						6,941	11,963	11,963	11,963	11,963
EBITDA						**1,561,233**	**1,683,757**	**1,723,422**	**1,899,100**	**1,940,100**
Depreciation and Amortization						1,002,108	1,002,108	1,002,108	1,002,108	1,002,108
EBIT=Operating Profit						559,125	681,649	721,314	896,992	937,992
Adjusted Income Taxes	**42%**					234,833	286,293	302,952	376,737	393,957
(Adjusted) Net Income						324,293	395,357	418,362	520,255	544,036
OCF=Operating Cash Flow						**1,326,401**	**1,397,465**	**1,420,470**	**1,522,363**	**1,546,144**
Capital Expenditures (CAPEX)					5,010,540					
Project Net Cash Flow					**- 5,010,540**	**1,326,401**	**1,397,465**	**1,420,470**	**1,522,363**	**1,546,144**
Cumulated Net Project Cash Flows					- 5,010,540	- 3,684,139	- 2,286,675	- 866,204	656,159	2,202,303

Project Discount Rate	**8.70%**			
Net Present Value (=NPV)	**607,656.55**			
Internal Rate of Return (=IRR)	**13.12%**			
Profitability Index	**1.12**			
Value Creation Index	**12.13%**			
Accounting Pay Back	**2030.57**	**3**	**Years**	**7** **Months**

Figure K13 – Financial viability.

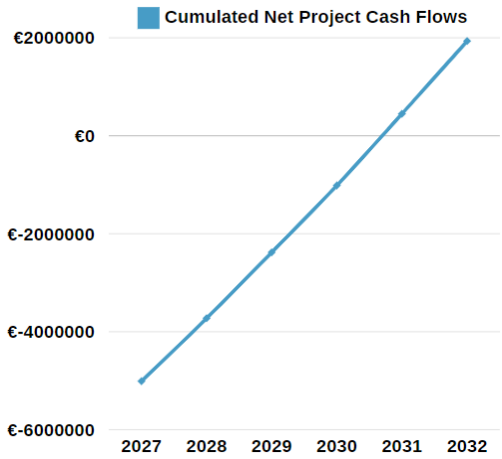

Figure K14 – Cumulative net project cash flows per year. For financial viability, the year 2027 was considered as year 0, with the sum of all investments from 2024 to 2027.

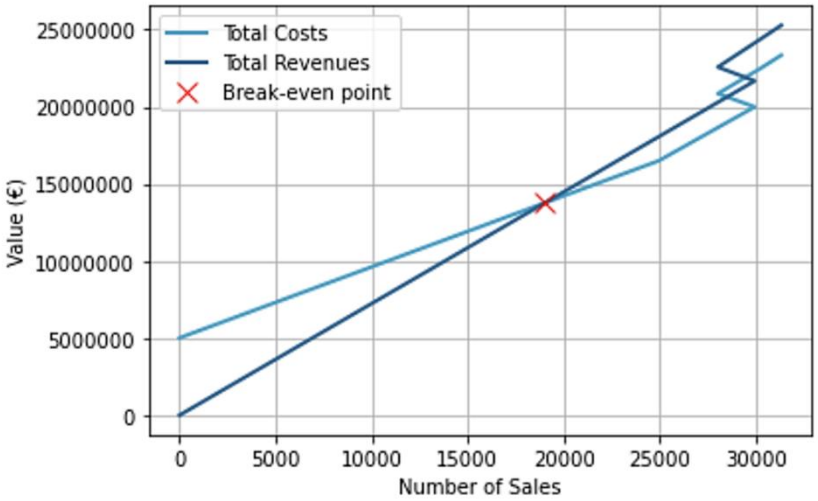

Figure K15 – Cost-volume-profit (CVP) graph.