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Point-of-Care and OECT Technologies for Biomarker Detection: Experimental Evaluation

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Chapter 1

Introduction

1.1 Context of Solute Analysis in the Biomedical Field

A biomarker can be defined as a quantifiable biological indicator that offers insights into normal physiological functions, disease states, or reactions to a stimulus or treatment. Biomarkers might include molecular, structural, imaging, or physiological features and, based on their intended use, could provide data for disease diagnosis, condition tracking, outcome prediction, treatment efficacy assessment, or response monitoring [1].

Such solutes may be proteins or small molecules in the blood that carry important information about a patient's clinical status [2,3].

The identification of biomarkers has become increasingly essential in the diagnosis, monitoring and prediction of many diseases in the last decade. In this context, a variety of specific biomarkers have been identified that can tell us important information about the function of organs and the course of disease [1,2,3].

In addition to the analytical reliability of biomarker measurements, the time required to obtain a result is particularly important in clinical practice. Rapid biomarker analysis can support earlier diagnosis and patient monitoring and can reduce the time between sample collection and clinical decision-making. This becomes particularly relevant when timely information is required to guide patient management, since the turnaround time associated with conventional laboratory analysis may delay clinical assessment and subsequent treatment [2,4]. The need to obtain reliable analytical information within shorter time

frames has therefore encouraged the development of rapid and portable analytical systems that can operate closer to the patient [3,4].
There has also been growing interest in rapid, portable, non-invasive analytical methods that might allow data to be generated rapidly, also in non-clinical environments.
This has prompted the development of alternative tools, including electrochemical sensors and rapid immunochromatographic devices, which are increasingly considered as effective alternatives to traditional laboratory methods. Ideally, these approaches should enable real-time measurements, require only small sample volumes and provide clear and reliable detection, ensuring accurate and reproducible results [2,3].
The miniaturization and optimization of such devices, aimed at minimizing key parameters such as measurement time, has recently been a subject of interest [3–5]. From these, Point-of-Care (POC) devices represent one of the most attractive solutions: they can provide substantially improved disease management since they can detect the presence of biomarkers in real-time with minimal sample quantities, directly at the point of care [2–4]. This reduces the need for centralized laboratory testing and enables more immediate clinical decision-making [4]. The selection of the most appropriate POC device still needs to be cautiously considered in terms of reliability, analytical stability and simplicity of use.

1.2 Detection Methods in Rapid Tests for selected Biomarkers

In the present work, we address specific small molecules (urea, creatinine) and proteins (CRP, IL-6, myoglobin, cystatin C) and discuss various technologies, typically encompassing enzymatic and electrochemical, optical and immunological methods, each offering different performances in terms of sensitivity, selectivity, response time, practical applicability and integration with other microfluidic functions [2,3,6].
The biomarkers chosen are molecules with different biological roles and consequently different clinical importance. Creatinine comes from creatine metabolism. It is eliminated mainly through renal filtration and is often measured to assess renal function. Urea, on the other hand, is the metabolic product of nitrogen. It is commonly used as a marker for the control of hemodialysis. It is also measured to assess renal function [2,3].
Cystatin C is another indicator of renal function. Its blood concentration indicates glomerular filtration and is less influenced by variables such as muscle mass, sex and race than creatinine, making it especially helpful [2]. CRP is frequently evaluated for diagnosing and tracking of inflammatory disorders: it is an acute-phase protein whose concentration increases in response to inflammation and tissue damage [6]. IL-6, a cytokine involved in immunological

and inflammatory responses, is an important biomarker in diseases with systemic inflammation, such as sepsis [7]. Myoglobin, a small oxygen-binding protein found in skeletal and cardiac muscle, is rapidly released into the bloodstream after muscle damage. Myoglobin has historically been used as an early indicator of myocardial injury because of its fast kinetics. However, its lack of cardiac specificity limits its clinical applicability [8]. When combined, these biomarkers encompass inflammatory, muscular/cardiovascular and renal functions. As a result, they were regarded as typical targets for assessing various analytical techniques relevant to quick testing.

Enzymatic approaches are particularly well suited for small molecule analyses in biomedicine and are among the most widely employed detection methods. They are frequently combined with colorimetric or potentiometric detections [2,3]. Urea can be studied directly and indirectly. The hydrolysis of urea into ammonia (NH_3) and carbon dioxide is catalysed by the enzyme urease. Ammonium can be quantified by absorbance-based measurements generated from a coloured complex, or through further reactions. For example, ammonia reacts with substrates such as α-oxoglutarate in the presence of glutamate dehydrogenase (GLDH), converting NADH to NAD^+, which can be detected at 405 nm. Alternatively, the former ammonium ions (NH_4^+) can be potentiometrically determined with ion selective electrodes [3]. From an electrochemical point of view techniques are based on redox processes at electrode surfaces. A good, cheap catalyst for the oxidation of urea is nickel: in alkaline solution it is only at first oxidized to $Ni(OH)_2$ and then to NiOOH, which is the actual oxidising agent [3]. Sophisticated sensors can use iron-/graphene-based nanocomposites, with immobilized urease that would then change the electrical properties of material in the presence of urea. A detectable signal is produced by variations in conductivity or electron mobility [3,9].

Non-enzymatic indirect methods are also feasible, such as voltametric, based on the electrochemical generation of active chlorine species from Cl^- and further oxidation of urea to N_2 by them with the reduction of the content of active chlorine being synchronous with the concentration of analyte. Another possibility is chemiluminescence, where a reaction between urea and sodium hypobromite leads to the emission of light, this, for example, being increased by xanthene dyes [5]. Finally, FET-based sensors present another detection mode: urease immobilized onto the gate catalyses urea hydrolysis and changes the local electric field, thus the current between source and drain, allowing for accurate quantification [2].

As for creatinine, its detection is based on more elaborate series of enzymatic reactions. Creatininase (CAH) converts creatinine to creatine, which is then converted to sarcosine by creatine amidinohydrolase (CRH). The sarcosine is then oxidized to produce sarcosine oxidase (SAO) and hydrogen peroxide (H_2O_2). H_2O_2 reacts with the corresponding reagents generating a red complex. This can be detected using colorimetry. The H_2O_2 produced can be further oxidized on a platinum electrode to generate an electric current. This current is

directly proportional to the creatinine concentration. Potentiometric methods for ammonium ion determination based on generation and measurements with pH sensitive sensors are also employed [2,9].

Among conventional procedures, the Jaffe test, the oldest and most commonly used, is based on the creatinine—picric acid reaction in an alkaline solution that results in the formation of an orange complex, which can then be quantified by spectrophotometry [2]. High-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC-MS) are also other advanced methods and the highest resolution in complex mixtures can be achieved using two-dimensional chromatography (2D-LC) [2]. Further colour detection can be achieved by using gold or silver nanoparticles with changes in absorbance or solution colour directly proportional to analyte concentration [2]. Chemiluminescences has also been used with a reactive mixtures which containing luminol, peroxide and cobalt producing light which is proportional to the creatinine concentration [4].

In protein detection, antigen-antibody-specific interaction which relies on the mass production of monoclonal antibodies from cell immunology and recombinant technology has been widely used due to its specificity and reliability. This molecular specificity enables sensitive detection of target proteins, even in complex biological samples.

Among the immunological methods immunofluorescence is one of the most commonly used, in which the antibody against the antigen gets labelled with a fluorophore, or gets detected with the help of a secondary antibody which is labelled by a fluorophore. The amount of the released fluorescence being proportional to the target protein concentration was determined by FIA after sufficient washing steps of the bound particles. Another common approach is the immunoturbidimetric assay, which is based on the turbidity created in a clear solution by the antigen-antibody complex. It can then be measured by absorbance or light scattering and is very well-suited for automated systems.

Enzyme-Linked Immunosorbent Assay (ELISA) has long been acknowledged for its ability to bind specific proteins to a solid phase and to use labelled antibodies. Addition of the substrate leads to the enzyme-catalysed formation of a colour or a fluorescent product, which is proportional to the concentration of the antigen. Instead, Lateral Flow Immunoassay (LFA) technology provides a rapid and convenient approach suitable for Point-of-Care applications. These systems employ gold nanoparticles or fluorescent labels that generate a visible or measurable signal, typically detected with hand-held instruments [6].

In particular, in these methods, cystatin C is usually diagnosed by sandwich immunoassays in the ELISA or lateral flow assay format, in which the read-out is gold nanoparticle-based and prepared on site [2]. With LFA strips prepared with double-antibody systems, the colorimetric signal can still be visualized even at very low concentration of myoglobin. A high-providing clinical inflammatory protein, Interleukin-6, can be detected using LFAs amplified by fluorescent particles like europium or quantum dots, or using techniques based on Surface

Enhanced Raman Scattering (SERS) achieving exceptional sensitivity. Albumin can, finally, be determined with solid-phase immunoassays or LFA platforms that rely on the biotin–streptavidin interaction to produce a colorimetric signal directly proportional to the analyte concentration.

1.3 Objectives and Structure of the Work

The methodologies described above provide a wide variety of detection approaches for solutes in biomedical applications, meeting the ever-growing demand for sensitivity, speed and ease of operation under a wide range of clinical settings [2,3,6,7,9]. The combination of enzymatic, electrochemical and immunological approaches in miniaturized and portable devices represents an important step towards increasingly personalized and straightforward diagnostic strategies, potentially enabling analyses to be performed in close proximity to the patient.
Concomitantly, there is growing scientific interest in the development of bioanalytical methods based on emerging electrochemical technologies such as Organic Electrochemical Transistors (OECTs). These systems are attracting considerable attention because of their intrinsic signal amplification, operation at low voltages, compatibility with aqueous environments and potential integration with miniaturized electronic platforms for biosensing and diagnostic applications [10,11,12].
The aim of this thesis was to select and experimentally validate a commercial POC device, following a comprehensive and comparative study of different commercial bioanalytical strategies for the quantification of a subset of biomarkers.
Moreover, a preliminary study for the development and functionalization of an OECT was carried out for the detection and quantification of a target protein.
A market survey was carried out to identify and select suitable candidates among commercially available POC devices characterized by different features, operating principles and analytical targets.
In order to select the optimal POC device, multiple models were screened based on its analytical performance, including sensitivity and specificity, response time, ease of operation, portability and overall cost. Once purchased, the most suitable POC device was validated through mulitple experiments and compared against baseline data from the COBAS 8000 clinical laboratory (Roche Diagnostics, Careggi Hospital, Florence) to verify its precision and reliability in a true clinical environment.
In parallel, OECT-based systems were investigated through targeted functionalization and experimental optimization. Due to their ability to merge electrochemical detection with transistor-driven amplification into a compact

design, OECT sensors represent a highly promising tool for bioanalysis [10,11,12]. Consequently, the evaluation focused heavily on tracking signal stability, electrochemical response and measurement reproducibility.

Chapter 2

Point-of-Care (POC) Device Selection and Validation

2.1 Overview of POC Devices Available on the Market

In the clinical diagnostics, Point of Care Testing refers to any analysis performed near the patient's bed, outside the central laboratory, by appropriately trained clinical staff [5,16]. It is therefore recommended to carry out tests close to the patient's bed, where the immediate result may influence the therapeutic decision without the transfer of samples and processing steps typical of the centralized laboratory [5,6].

Advances in electronic miniaturisation, biosensoristics and reagent engineering have allowed the spread of small portable or bench-top devices operable by non-laboratory personnel with a time of response of a few minutes [5,16].

Experience from international laboratory networks, market analysis and operational observations show that POCs can shorten decision-making time, promote more targeted therapies and reduce inappropriate use of antibiotics, with positive impacts on overall care costs and patient satisfaction [5,6,17].

It is important to consider economic and technological factors. The growing diffusion of POC systems is driven by factors such as the reduction of time-to-result, the portability of systems and, above all, their integration with digital platforms and artificial intelligence algorithms [16,18,19].

POC systems are evolving from stand-alone devices to software-connected platforms that can transmit results in real time, enable centralized monitoring of instrument performance and integrate data into the digital clinical pathway [16,19].

Finally, the COVID 19 pandemic has broadened the use of rapid antigen tests, stimulating investments that have accelerated miniaturization, automation and

digital interpretation of results [18,20]. At the same time, the mass experience with rapid tests has increased public acceptance and application extension of lateral flow towards nutritional, infectious biomarkers and even time-dependent emergencies such as stroke or sepsis [18,20,21].

POCs are now being used or are being expanded in many areas. The most relevant clinical domains of use are related to sepsis and septic shock control or management of severe infections: rapid measurement of inflammatory and/or infectious biomarkers (e.g. procalcitonin, CRP) at triage or in critical care wards may support risk stratification, the appropriateness of starting or stopping antibiotic therapy and monitoring response [17]. They are especially useful in intensive care, in fact installing POC in emergency areas helps shorten the time between sample collection and result availability for critical parameters (inflammatory markers), contributing to faster decision-making and reducing the duration of stay in critical areas [6,17].

The POC market is heterogeneous and can be organized into large technological families. The following tests were considered in this work: qualitative single-use lateral flow tests/test strips; quantitative immunofluorimetric or immunochromatographic analysers by cartridge; dry chemistry systems or multiparametric "rotor disc" integrating centrifugation and photometry; bench-mounted integrated immunometric platforms with specimen handling [16,18,21].

When defining the criteria for acquiring a Point of Care platform, the first element considered was the availability of biomarkers such as Albumin, Urea, Creatinine, C-reactive protein (CRP), Interleukin-6, Cysteine, Myoglobin, B2microglobulin, Calcidiol/25OHVD, cardiac markers and D dimer.

This requirement was accompanied by the need for the system to guarantee adequate analytical performance (in terms of precision, accuracy and quantification limits) with respect to the decision-making cut-offs reported in the literature and codified in the internal protocols. This was done to avoid areas that are difficult to interpret [16,22].

Another crucial aspect concerned the type of sample: priority was given to the use of whole, venous or capillary blood, without centrifugation, in order to compress the analysis times and reduce the infrastructural and technical personnel requirements typical of the central laboratory [5,16].

All these choices were weighed within an analysis of the total purchase cost of the instrument and reagents. Finally, regulatory compliance with European legislation (CE marking) and the availability, by the supplier, of a complete set of documents (technical dossier, clinical evidence, post-marketing surveillance plans) useful to guarantee continuity of use were considered essential [23].

In summary, the selection of the POC instrument was the outcome of an integrated assessment in which clinical needs, analytical requirements, operational logistics, digital interoperability, economic sustainability and regulatory compliance were considered as inseparable dimensions of the same decision problem [5,16,19,23]. For this reason, attention was limited to the following POC devices.

An initial market survey was conducted to compare commercially available POC systems with different levels of portability, analytical principles and operating

characteristics. The main technical and economic features considered during this preliminary screening are summarized in Table 1.

Table 1. Main characteristics of the POC devices considered during the preliminary market survey

Device	Analytical principle / detection	Sample	Analysis time	Memory	Dimensions (mm)	Weight	Price*	Main characteristics
abioSCOPE (Abionic)	Nanofluidic biosensors; optical detection using two lasers	Few µL depending on the assay; ferritin capsule: 50 µL lithium-heparin whole blood	Ferritin: 7 min	20,000 tests	281 × 323 × 187	8 kg	—	Fully automated system based on nanofluidic IVD capsules and a four-step workflow. Ferritin assay: 20–600 ng/mL measurement range and 20 ng/mL sensitivity
Getein 1100	Fluorescence immunochromatography; optical scanning followed by signal conversion	Serum, plasma, whole blood, urine, fingertip blood and other assay-dependent samples	3–15 min	10,000 results	261 × 241 × 115	2 kg	€3,228.74	Single-channel quantitative immunofluorescence analyzer; SD card, USB and Ethernet connectivity
Getein 1180	Immunofluorescence	Assay-dependent	3–15 min	100,000 tests	344 × 409 × 193	9 kg	€3,228.74	Higher-capacity immunofluorescence platform
Getein 1160	Immunofluorescence; optical detection based on antigen–antibody interaction	Assay-dependent	3–15 min	100,000 tests	299 × 276 × 152	4 kg	€1,675.57	Quantitative immunofluorescence platform
Getein 208	Dry clinical chemistry + immunofluorescence	Serum, plasma, whole blood, capillary blood, urine and other assay-dependent samples; 10–200 µL depending on assay	3–15 min	>10,000 tests	160 × 80 × 24	256 g	€1,032.02	Hand-held system; SD-card calibration; USB/Bluetooth/Wi-Fi connectivity; supports both dry chemistry and immunofluorescence
ESEQuant Flex	Optical reader for colorimetric/lateral-flow assays	Dependent on the lateral-flow assay employed	Assay-dependent	—	180 × 160 × 145	1.2 kg	—	Rapid reader designed for quantitative/qualitative evaluation of lateral-flow tests

**Prices correspond to the commercial quotations considered during the preliminary selection and therefore refer to the conditions available at the time of the market survey.*
Sources: [24–29].

2.2 Selection and Purchase

The Getein 1100 is an immunofluorescence-based quantitative analyzer designed for reading disposable test cartridges dedicated to a wide range of biomarkers, including cardiovascular, renal, inflammatory, fertility and bone metabolism markers. The analytical principle combines immunochromatographic migration with fluorescence detection: the target analyte interacts with specific antibodies within the test cartridge and the resulting fluorescent signal is optically scanned by the analyzer and converted into a quantitative result related to the concentration of the analyte [25].
From an operational point of view, the protocols indicate the use of different anticoagulants, including heparin, EDTA and citrate, depending on the specific assay and recommend analysis within defined time intervals after collection for whole blood samples, with specific storage conditions for serum, plasma and urine. The declared intra-run and inter-run precision varies according to the specific assay and should therefore be considered in relation to the corresponding test kit [25]. Platform variants such as Getein 1160 and Getein 1180 provide alternative configurations and higher-throughput capabilities [26,27].
Several studies have investigated the application of the Getein 1100 in different clinical settings, providing useful information both on its practical applicability and on the analytical limitations that need to be considered when comparing results obtained from different POC platforms.
In their study, Rizo Topete et al. evaluated the utility of microalbuminuria and urinary neutrophil gelatinase-associated lipocalin (uNGAL) as indicators of acute kidney injury (AKI) in COVID-19 patients. Fifty SARS-CoV-2-positive patients with serum creatinine <1.0 mg/dL were the subjects of the analysis. Urine samples were analyzed using the Getein 1100 quantitative immunofluorescence platform. The results suggested a possible association of these urinary biomarkers with AKI, although their predictive role required further investigation. Importantly for the present work, Getein 1100 was used as the analytical platform but was not designed as a method-comparison or analytical-validation study; consequently, it does not provide direct evidence of the accuracy or agreement of the device relative to a laboratory reference method [30].
In the study by Çelik, the analytical performance of Nano-Ditech and Getein 1100 was compared for the quantitative measurement of cardiac troponin I (cTnI), CK-MB and myoglobin. This work included 100 patients, 53 men and 47 women, presenting with chest pain. Lithium-heparin plasma samples were analyzed with both systems. The comparison showed marked discordance between the two devices, with a correlation coefficient of 0.023 for troponin I, 0.130 for CK-MB and no significant correlation for myoglobin. The author attributed these discrepancies, among other factors, to differences in the antibodies used by the assays and to the

heterogeneous molecular forms of cardiac biomarkers. Since neither instrument represented an established laboratory reference method, the results do not demonstrate the superiority of either platform, but instead highlight the importance of method comparison and standardization when results from different POC systems are interpreted [31].

Further information on the analytical performance of Getein 1100 was provided by Li et al., who compared four commercial POC assays for procalcitonin measurement: AQT90 FLEX, mLabs, Finecare and Getein 1100. Whole blood and plasma measurements were evaluated for repeatability, linearity, accuracy and diagnostic concordance against the Elecsys BRAHMS PCT assay performed on the Cobas e602 platform. AQT90 FLEX showed the most consistent analytical performance across matrices and PCT concentrations, whereas each of the other POC systems showed at least one analytical limitation. Getein 1100 showed adequate precision particularly in plasma at high PCT concentrations, while its accuracy in the method-comparison analysis was considered adequate. Finecare showed excellent diagnostic concordance with the reference method at the 0.25 ng/mL cut-off and, together with Getein 1100 and AQT90 FLEX, at the 0.5 ng/mL cut-off. Overall, the study demonstrates that acceptable diagnostic concordance does not necessarily imply equivalent analytical performance across devices, matrices and concentration ranges [32].

The potential use of uNGAL in POC-oriented settings was also investigated by Roy et al. in a cohort of 288 critically ill children aged between 3 months and 12 years who were admitted to a pediatric intensive care unit without AKI at admission. Fresh non-centrifuged urine was used for uNGAL measurement. The study showed that uNGAL could contribute to the early identification of severe AKI in critically ill pediatric patients. However, the primary objective of the investigation was the diagnostic performance of the biomarker rather than the analytical validation of the analyzer itself; therefore, the study provides evidence for the clinical usefulness of uNGAL but cannot be considered direct evidence of the analytical accuracy of Getein 1100 [33].

Among the devices considered during the selection process, Finecare™ FIA Meter Plus (FS113) was of particular interest because it is a compact quantitative fluorescence immunoassay platform capable of providing results within a short analytical time and offering a broad test menu covering inflammatory, cardiovascular, metabolic and other biomarkers. As with the Getein systems, the analytical workflow is based on specific test cartridges and immunofluorescence detection, while sample requirements, anticoagulants and storage conditions depend on the individual assay.

The analytical performance of Finecare was specifically investigated by Ismail et al. for procalcitonin measurement in critically ill septic patients. Forty samples were analyzed by Finecare™ FIA Meter Plus (FS113) using whole blood and compared with the Elecsys BRAHMS PCT assay performed on the Cobas e411 laboratory platform using plasma. The study reported a strong relationship between the measurements obtained with the two systems and supported the potential use of Finecare as an alternative PCT measurement approach in an intensive-care setting. This study is particularly relevant to the device-selection process because, unlike

studies in which the POC analyzer was used only as a measurement tool, it directly compared Finecare with an established automated laboratory immunoassay [34].
Finecare has also been employed in clinical studies in which analytical validation was not the primary objective. In the study by Sordi et al., 69 overweight volunteers with different post-COVID-19 clinical conditions were initially enrolled in a multidisciplinary intervention program, of whom 35 completed the entire protocol. Biochemical parameters including glucose, lipid profile, liver enzymes and CRP were measured using the URIT 8021 system, whereas HbA1c was determined using Finecare FIA Meter Plus. The study demonstrated changes in several biochemical parameters following the intervention, but it did not compare Finecare with a reference analytical platform or specifically evaluate its precision, accuracy or agreement. Consequently, it supports the practical use of the instrument for HbA1c measurement within a clinical research protocol but cannot, by itself, establish its analytical reliability [35].
Another system considered during the preliminary selection was the Seamaty SD1, a fully automated dry-chemistry biochemical analyzer that integrates sample processing, disposable reagent rotors and photometric/turbidimetric detection. The platform requires relatively small sample volumes and provides multiple biochemical measurements from a single rotor, thereby representing a different technological approach from the immunofluorescence-based systems considered above.
In the study by Lu et al., the analytical performance of the Seamaty SD1 for cholinesterase measurement was compared with that of the Cobas 6000 c501 automated biochemical analyzer. Precision, linearity and method agreement were evaluated. Both systems showed coefficients of variation below 3%, with slightly lower CV values for the SD1 in the reported precision experiments. The linearity correlations were also high ($r = 0.9999$ for SD1 and $r = 0.9998$ for Cobas c501) and the method-comparison analysis showed small biases and good correlation between the two systems. The authors therefore concluded that both detection systems provided good precision and linearity and that the results were sufficiently consistent to support the use of the SD1 for cholinesterase determination [36].
The economic evaluation performed during the present work also considered the purchase and management costs of the different platforms. For the Seamaty SD1, the commercial quotation was of approximately €6,590, with the possibility of a three-year rental arrangement of approximately €3,500 per year. These values refer specifically to the commercial quotation available at the time of the market survey and should therefore not be interpreted as fixed manufacturer prices.
The FLI-100 was also considered during the preliminary market analysis. It is a compact immunofluorescence analyzer equipped with an integrated printer, touchscreen interface and data-storage functions. Although its test menu includes several clinically relevant biomarkers, the availability of assays for the main analytes of interest in this thesis, particularly urea and creatinine, was limited at the time of the evaluation. This reduced its suitability for the specific objectives of the present work.
Overall, the studies considered during the selection process indicate that commercially available POC systems can provide useful quantitative measurements and, in some applications, good agreement with established laboratory platforms.

However, their analytical performance cannot be assumed to be equivalent across biomarkers, sample matrices or concentration ranges. The comparison by Çelik showed substantial disagreement between two POC systems for cardiac biomarkers, while Li et al. demonstrated that different PCT analyzers may exhibit distinct patterns of precision, accuracy and diagnostic concordance [31,32]. Conversely, the comparison performed by Ismail et al. provided more favorable evidence for Finecare PCT measurements against an established laboratory immunoassay, while the results reported by Lu et al. supported the analytical consistency of the Seamaty SD1 for cholinesterase measurement [34,36]. Taken together, these findings indicate that the reliability of a POC device should not be considered an intrinsic property of the platform alone, but should be assessed in relation to the specific biomarker, biological matrix, analytical range and intended clinical application. This evidence, together with the availability of the biomarkers of interest, sample requirements, ease of use, analytical time and economic considerations, contributed to the subsequent selection of the device to be experimentally evaluated in this thesis.

2.3 Technical Features and Operating Principles of the Acquired POC

Finecare™ FIA Meter Plus was developed, designed, manufactured and sold by Guangzhou Wondfo Biotech Co., Ltd. Finecare™ FIA Meter Plus is a compact and portable fluorescence instrument used for the quantitative determination of concentrations of various analytes in human blood or urine [37].

Finecare™ FIA Meter Plus uses an LED as an excitation light source. The LED light hits the test strip that has been inserted into the instrument. This causes the fluorescence marker on the test strip to emit energy. The more energy the fluorescent marker emits, the stronger the signal [37].

After a sample mixed with the buffer is dispensed onto the test strip, the test strip is inserted into the Finecare™ FIA Meter Plus. The instrument measures the analyte concentration based on a pre-programmed calibration process. Finecare™ FIA Meter Plus can only accept test strips designed specifically for use with this instrument [37].

The instrument and test strips are for in vitro diagnostic use only. Finecare™ FIA Meter Plus can be used in a laboratory with Point-of-Care setting [37].

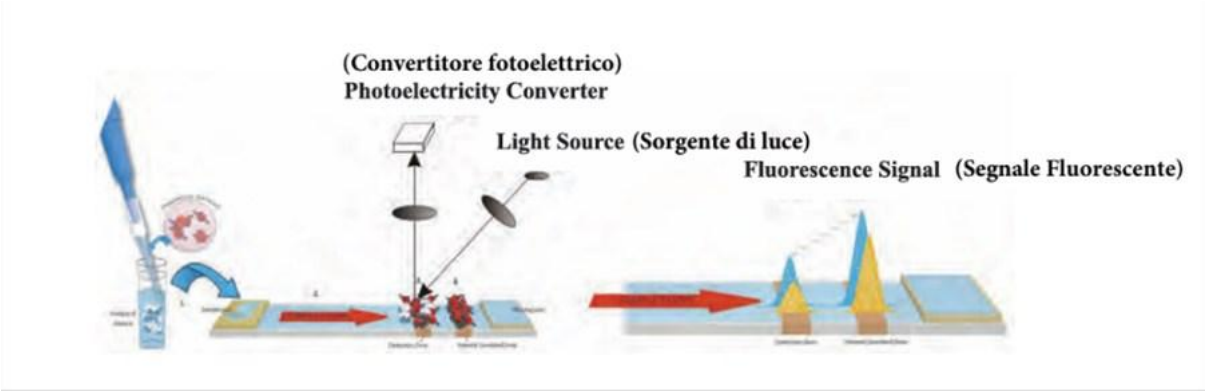


Figure 1: Finecare™ FIA Meter Plus (FS-113) fluorescence immunoassay analyzer.

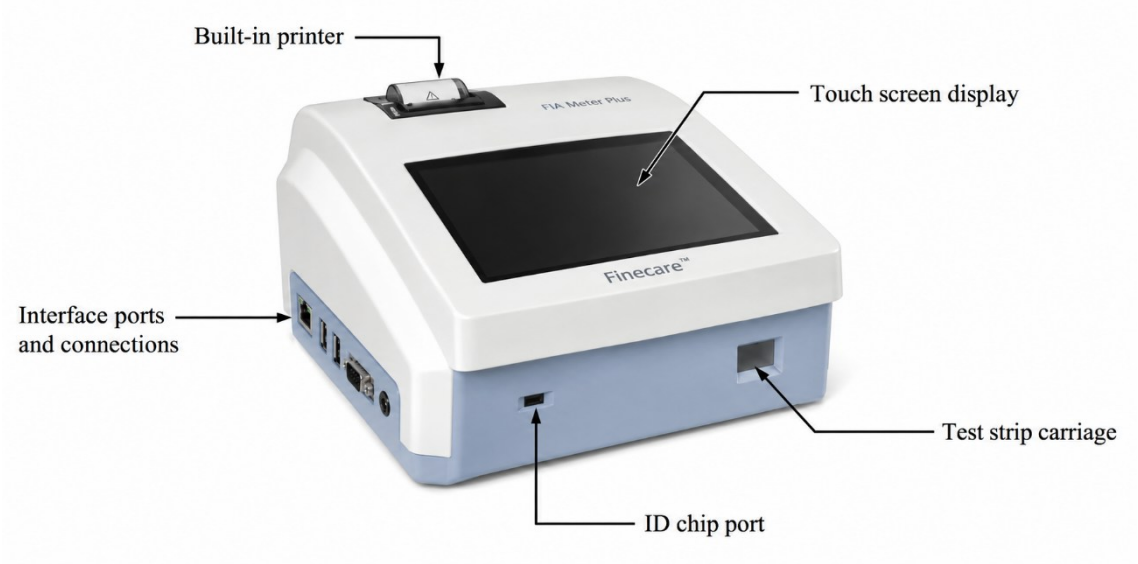


Figure 2: Finecare™ FIA Meter Plus analyzer and its main functional components.

The excitation source is LED. The excitation spectrum has a wavelength of λ = 470 nm, while the absorption spectrum corresponds to a wavelength of λ1 = 525 nm [37].

The type of sample used is whole blood, serum, plasma or urine, depending on the specific assay. The detector, on the other hand, is a photodiode. It has a memory capacity of more than 30,000 tests [37].

Depending on the analyte to be analyzed, different control strips are used and inserted inside the device [37].

It must be ensured that the sample liquids are completely absorbed before the test device is inserted into the instrument to avoid internal contamination of the meter. Additionally, the control strip should be stored away from sunlight and moisture when not in use [37].

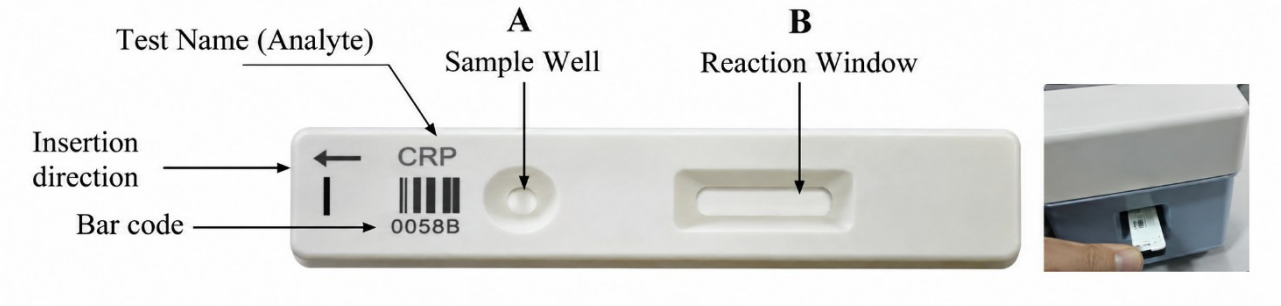


Figure 3: Finecare™ FIA Meter Plus test cartridge and its positioning in the analyzer.



Figure 4: Identification (ID) chip for the Finecare™ IL-6 Rapid Quantitative Test, used to load assay-specific calibration information into the analyzer.

Test strips and chip IDs are packaged separately from the instrument and include instructions for performing specific tests [37].

In our case, the Finecare™ IL-6 Rapid Quantitative Test was used for testing [38].

IL-6 is an inflammatory factor that is a key component of the inflammatory mediator network and plays an important role in the inflammatory response. IL-6 is produced earlier than CRP and PCT, induces CRP and PCT and its concentration is related to the severity of inflammation and sepsis [38,39,40,41].

The Finecare™ IL-6 Rapid Quantitative Test is based on fluorescence immunoassay technology. The Finecare™ IL-6 Rapid Quantitative Test uses a sandwich immunodetection method. Whole blood, serum or plasma is mixed with the detection buffer containing fluorescence-labeled detector antibodies against IL-6 [38].

When the sample is added into the sample well of the test cartridge, the fluorescence-labeled detector IL-6 antibodies on the sample pad bind to the analyte in the sample and form immune complexes. As the complexes migrate on the nitrocellulose matrix of the test strip by capillary action, the complexes of detector antibodies and IL-6 antigens are captured by IL-6 antibodies that have been immobilized on the test strip. Thus, the more IL-6 antigens present in the blood

specimen, the more complexes accumulate on the test strip. The fluorescence signal intensity of the detector antibodies reflects the amount of captured IL-6 [38].
The test can be performed with serum, plasma or anticoagulated whole blood. It is recommended that specimens should be tested immediately [38].

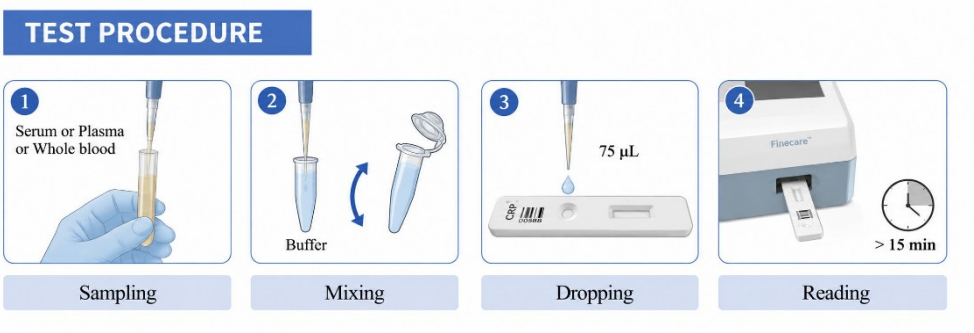


Figure 5: Main steps of the Finecare™ IL-6 Rapid Quantitative Test procedure

There are two test modes for the Finecare™ FIA System: Standard Test mode and Quick Test mode [37,38].
Standard Test consists of inserting the test cartridge onto the test cartridge holder of the Finecare™ FIA System immediately after adding the sample mixture, then pushing the test strip into the instrument carriage immediately after the sample has been completely absorbed. The analyzer serves as an incubation location for the reaction for a predefined duration. The test result appears on the screen [37,38].
Quick Test mode implies the immediate detection of the signal, requiring a "manual" incubation. In particular, after the sample mixture is added to the sample well, the cartridge is left at room temperature for 15 minutes and is then inserted into the Finecare™ FIA System, which immediately scans the sample-loaded test cartridge [38].
The Finecare™ FIA System calculates IL-6 test results automatically and displays the exact concentration of IL-6 on the screen with one decimal place, expressed in pg/mL [38]. The normal reference value reported by the manufacturer is ≤10 pg/mL; however, the manufacturer recommends that each laboratory establishes its own reference range according to the specific situation [38].
There may be limitations of the procedure, such as false-positive or false-negative results [38].
Reasons for false-positive results could include non-specific components, such as heterophilic antibodies and antigenic structural analogues in the blood, which bind directly to the antibody [38].
Possible reasons for false-negative results include unknown components that prevent the antigenic determinant from binding to the antibody; additionally, the IL-6 antigen may be broken down and therefore cannot be recognized by the antibody [38].

The accuracy of the device reported on the datasheet refers to a comparative study in which 110 clinical samples were tested using the Finecare™ IL-6 Rapid Quantitative Test and the Roche IL-6 Reagent Kit. The reported correlation coefficient between the two methods was R = 0.980 [38].
The specifications report an assay range of 3–4000 pg/mL and a detection limit ≤3 pg/mL. Linearity was evaluated over the concentration range from 3 to 4000 pg/mL, with a reported correlation coefficient of R ≥0.9900. Within-run precision was determined using two concentrations of IL-6 precision controls with one batch of tests, while between-run precision was determined using two concentrations of IL-6 precision controls with three batches of tests. In both cases, the reported coefficient of variation was CV ≤15% [38].

2. 4 POC Validation: Protocol and Rphaseesults

The experimental assessment of the Finecare™ FIA Meter Plus for interleukin-6 (IL-6) determination was carried out in two stages. Preliminary tests were performed to analyze the device using different biological matrices. Based on the observations obtained, a second experimental phase was subsequently conducted. This phase involved the quantitative assessment of recovery and repeatability.
The first experimental phase provided an initial evaluation of the device's operation and response under different measurement conditions. Analyses were therefore performed on whole blood, plasma obtained from the same sample and plasma subjected to freezing and subsequent thawing. In parallel, the Standard and Quick analysis modes available on the Finecare™ system were compared.
An initial series of measurements was performed using whole blood obtained from a buffy coat bag. Four consecutive IL-6 determinations were carried out on the sample: the first using Standard mode and the following three using Quick mode.
The Standard measurement yielded an IL-6 concentration of 4.4 pg/mL. The three subsequent measurements performed in Quick mode yielded values of 4.2, 3.8 and 3.4 pg/mL, respectively.
Plasma was subsequently isolated from the same sample by two centrifugation cycles and further measurements were performed. In this case, Standard mode yielded an IL-6 concentration of 3.1 pg/mL, whereas the measurement performed in Quick mode gave a result below the detection limit of 3 pg/mL.
These initial results showed IL-6 concentrations close to the lower limit of the measurement range of the system and revealed a slight difference between the result obtained from whole blood and that obtained from plasma isolated from the same sample. Given the low endogenous IL-6 concentration in the sample, these experiments were primarily exploratory and were not sufficient to provide a quantitative assessment of device accuracy.
A subsequent experiment was performed on plasma previously isolated from the same original buffy coat bag. The plasma was stored at −20 °C for 20 days and subsequently thawed at room temperature before analysis.

Following the freeze–thaw cycle, the IL-6 concentration measured in Standard mode was 3.6 pg/mL, while the two consecutive measurements performed in Quick mode both yielded a value of 3.4 pg/mL.
The results therefore remained within the same concentration range observed before freezing. Under the adopted experimental conditions, no substantial change in the measured concentration was observed following storage at −20 °C and subsequent thawing. However, given the limited number of measurements and the proximity of the values to the lower measurement limit of the device these results were considered only indicative and did not offer indications on the stability of the IL-6.
Since the concentrations initially detected were very low, subsequent experiments involved the addition of known amounts of IL-6 to the plasma. The goal was to increase the analyte concentration in a controlled manner.
In the first experiment, 350 µL of sample was spiked with 1 pg of IL-6, resulting in an expected concentration of 6.25 pg/mL, as reported in the experimental protocol. However, the determination performed in Standard mode yielded a result below 3 pg/mL. The measured concentration was therefore considerably lower than the expected value.
A second experiment was then performed spiking 275 µL of sample with 5 pg of IL-6, which would result in an expected concentration of 24.4 pg/mL.
The first determination, performed in Standard mode, yielded a concentration of 20.6 pg/mL. Subsequent measurements performed in Quick mode yielded concentrations of 23.6, 26.9, 28.2, 29.9 and 31.5 pg/mL.
This experiment therefore showed a different behaviour compared with the first spiking test. In particular, when the IL-6 concentration was increased to a level further from the lower measurement limit, the device was able to clearly detect the increase in analyte concentration. The standard measurement was close to the nominal value of 24.4 pg/mL. Rapid measurements showed progressively increasing values, ranging from 23.6 to 31.5 pg/mL.
The trend observed in consecutive Quick measurements indicated the need for further investigation of device behavior using a more controlled approach.
An additional series of experiments was performed using plasma that had previously been stored at −20 °C for 20 days and subsequently thawed at room temperature.
In the unspiked sample, an IL-6 concentration of 3.0 pg/mL was measured in Standard mode, while Quick mode yielded a value below 3 pg/mL, once again confirming the very low endogenous concentration of the analyte.
Subsequently, 425 µL of sample was spiked with 2.5 pg of IL-6, resulting in an expected concentration of 8.88 pg/mL, as reported in the experimental protocol. In this case, both the Standard measurement and the two Quick determinations yielded an IL-6 concentration of 3.3 pg/mL.
This experiment therefore also showed a marked underestimation of the nominal concentration. The result was consistent with that observed in the previous experiment at 6.25 pg/mL and suggested limited quantitative recovery of small amounts of added IL-6 under the experimental conditions used.

Table 2. Summary of preliminary Finecare™ IL-6 measurements.

Experiment	Sample	Nominal concentration (pg/mL)	Standard measurement (pg/mL)	Quick measurement, mean (pg/mL)
1	Unspiked whole blood	/	4.4	3.8 ± 0.4
1	Unspiked plasma	/	3.1	< 3
2	Unspiked plasma after freeze–thaw	/	3.6	3.4
3	Spiked plasma	6.25	< 3	—
4	Spiked plasma	24.4	20.6	28.02
5	Unspiked plasma after freeze–thaw	/	3.0	< 3
5	Spiked plasma after freeze–thaw	8.88	3.3	3.3

Overall, the preliminary tests provided useful information for the design of the subsequent validation phase.

Measurements performed on unspiked samples showed endogenous IL-6 concentrations of approximately 3–4 pg/mL, i.e., close to the lower measurement limit of the system. Consequently, these preliminary measurements were not considered suitable for a reliable quantitative assessment of accuracy.

The spiking experiments revealed a concentration-dependent response. At relatively low expected concentrations of 6.25 and 8.88 pg/mL, the device returned values close to or below 3 pg/mL, indicating marked underestimation. In contrast, in the sample with an expected concentration of 24.4 pg/mL, the Finecare™ system clearly detected the increase in IL-6.

Another relevant observation concerned the behaviour of consecutive measurements. Although Standard and Quick measurements were generally of the same order of magnitude, a progressive increase or decrease in the measured value was observed during consecutive Quick determinations of different samples. Since this phenomenon was not systematically observed across all experiments, no definitive conclusion could be drawn; nevertheless, it provided further justification for a more controlled characterisation of the system.

The preliminary findings therefore highlighted the need to extend the concentration range under investigation and to further standardise sample preparation. For this purpose, a second experimental phase was designed in which samples with known nominal IL-6 concentrations were prepared using two different matrices, plasma and saline solution. For each concentration level, one measurement was performed in Standard mode followed by three consecutive measurements in Quick mode. This second protocol allowed accuracy to be assessed more systematically through the calculation of recovery and bias, while also providing an initial indication of the repeatability of Quick measurements.

A stock IL-6 solution was prepared in 0.9 % (w/v) NaCl, supplemented with 0.1% BSA. Stock IL-6 solution was used at a concentration of 10 pg/µL.
The stock IL-6 solution was used to spike the matrices employed in the validation experiments.
Plasma obtained as described above was used as the matrix for the preparation of the following IL-6 test solutions: 200, 100, 50, 20 and 0 pg/mL. The 0 pg/mL sample consisted of unspiked plasma and was used as the control.
Saline solution was used as the matrix for the preparation of the following IL-6 test solutions: 200, 50, 20 and 0 pg/mL. The 0 pg/mL sample consisted of unspiked saline solution and was used as the control.
The prepared samples were analysed using the Finecare™ FIA Meter Plus with the IL-6-specific assay. A sample volume of 75 µL was used for each concentration level.
The sample matrix was selected on the device before each measurement. The "plasma/serum" setting was selected for samples prepared in plasma, whereas the "urine" setting was used for samples prepared in saline solution.
For each concentration, the same sample was subjected to four consecutive determinations. The first determination was performed using Standard (ST) mode, while the following three determinations were performed using Quick mode. The latter provides a result in a shorter time than the Standard procedure.
Information was obtained on two distinct aspects: the comparison between nominal and measured concentrations allowed an assessment of analytical accuracy; measurements performed in Quick mode allowed a preliminary assessment of the repeatability of the measurement.
Accuracy was expressed in terms of recovery percentage and distortion percentage. Recovery represents the concentration obtained experimentally as a percentage of the predicted concentration: a value of 100% would indicate an ideal agreement between the nominal and measured concentrations. Percentage bias expresses the relative deviation of the measured result from the nominal value: negative values indicate underestimation of the concentration, whereas positive values indicate overestimation.
Results of the measurements are summarized in the following table.

Matrix	Nominal concentration (pg/mL)	Standard measurement (pg/mL)	Quick measurement, mean (pg/mL)	Recovery (%)	Bias (%)
Saline solution	0	< 3	< 3	/	/
Saline solution	20	< 3	< 3	0	−100
Saline solution	50	3.5	3.5	7.00	−93.00
Saline solution	200	36.5	38.5	18.25	−81.75
Spiked plasma	0	< 3	< 3	/	/
Spiked plasma	20	10.0	10.0	50.00	−50.00
Spiked plasma	50	37.0	38.17	74.00	−26.00
Spiked plasma	100	65.5	69.4	65.50	−34.50

Table 3. Finecare™ IL-6 measurements in saline solution and spiked plasma.

Measurements performed on samples prepared in saline solution revealed a marked discrepancy between nominal concentrations and those determined by the Finecare™ system.

The 0 pg/mL control sample yielded values below 3 pg/mL in both Standard mode and all three Quick measurements. Similarly, for the sample with a nominal concentration of 20 pg/mL, the instrumental measurements were below 3 pg/mL. In order to calculate the accuracy, the recorded value was considered to be 0 pg/mL. This corresponds to 0% recovery and -100% bias.

For the nominal 50 pg/mL sample, a concentration of 3.5 pg/mL was obtained in Standard mode, corresponding to a recovery of 7% and a bias of −93%. The mean value of the three Quick determinations was also 3.5 pg/mL.

Underestimation was also evident at the highest nominal concentration. For the 200 pg/mL sample, Standard mode yielded a concentration of 36.5 pg/mL, corresponding to a recovery of 18.25% and a bias of −81.75%. The mean value of the three Quick determinations was 38.5 pg/mL.

Considering the non-zero nominal concentrations, the mean recovery reported for Standard measurements in saline solution was 8.42%, while the mean bias was −91.58%. These results therefore indicate a marked and systematic underestimation of the IL-6 concentration in samples prepared in saline solution.
Despite the poor agreement with nominal concentrations, the three consecutive measurements performed in Quick mode showed good agreement with one another. For the 50 pg/mL sample, the same result was obtained in all three determinations, whereas for the 200 pg/mL sample the mean of the Quick measurements was 38.5 pg/mL, with a standard deviation of approximately 0.8 pg/mL and a coefficient of variation of approximately 2.08%. This finding suggests that the observed underestimation was not accompanied by substantial variability between consecutive measurements.
A different behaviour was observed for samples prepared in plasma. In this matrix, the concentrations quantified by the device were also lower than the nominal values; however, the degree of underestimation was considerably lower than that observed in saline solution.
The control sample with a nominal concentration of 0 pg/mL yielded values below 3 pg/mL in all determinations.
For the nominal 20 pg/mL sample, Standard mode yielded a concentration of 10 pg/mL, corresponding to 50% recovery and −50% bias. The mean value of the three Quick determinations was 10.0 pg/mL.
At the nominal concentration of 50 pg/mL, a value of 37 pg/mL was obtained in Standard mode, corresponding to a recovery of 74% and a bias of −26%. The mean value of the three Quick determinations was approximately 38.17 pg/mL.
Finally, for the nominal 100 pg/mL sample, Standard mode yielded a concentration of 65.5 pg/mL, corresponding to a recovery of 65.5% and a bias of −34.5%. The mean value of the three Quick determinations was 69.4 pg/mL.
Considering the three concentration levels, the mean recovery for Standard measurements was 63.17%, with a mean bias of −36.83%. Although these values confirm systematic underestimation of the nominal IL-6 concentration, the accuracy obtained in plasma was markedly higher than that observed in saline solution.
For plasma samples, the three consecutive Quick measurements also showed relatively limited variability. The coefficients of variation calculated from the three Quick replicates were 1.73%, 0.92% and 2.46% for nominal concentrations of 20, 50 and 100 pg/mL, respectively. The limited dispersion of the measurements obtained in quick mode therefore indicates good repeatability of consecutive determinations despite the systematic deviation from the nominal concentrations.
Overall, the results showed underestimation of the nominal IL-6 concentrations. This effect was particularly pronounced in samples prepared in saline solution, whereas better agreement between expected and measured concentrations was observed in plasma samples. The differences between the two matrices therefore suggest that sample composition has a significant influence on the analytical response of the system.
The experiments performed should be regarded as a preliminary experimental assessment of Finecare™ performance under the conditions adopted rather than as a complete analytical validation of the method. Nevertheless, they allowed the

response of the device to different IL-6 concentrations to be characterised, highlighted its behaviour in different matrices and provided an initial indication of measurement repeatability. These findings therefore provide the basis for the subsequent overall assessment of the device, in which the observed analytical performance, the characteristics of the Standard and Quick operating modes, usability and the economic implications of its use as a Point-of-Care system are considered together.

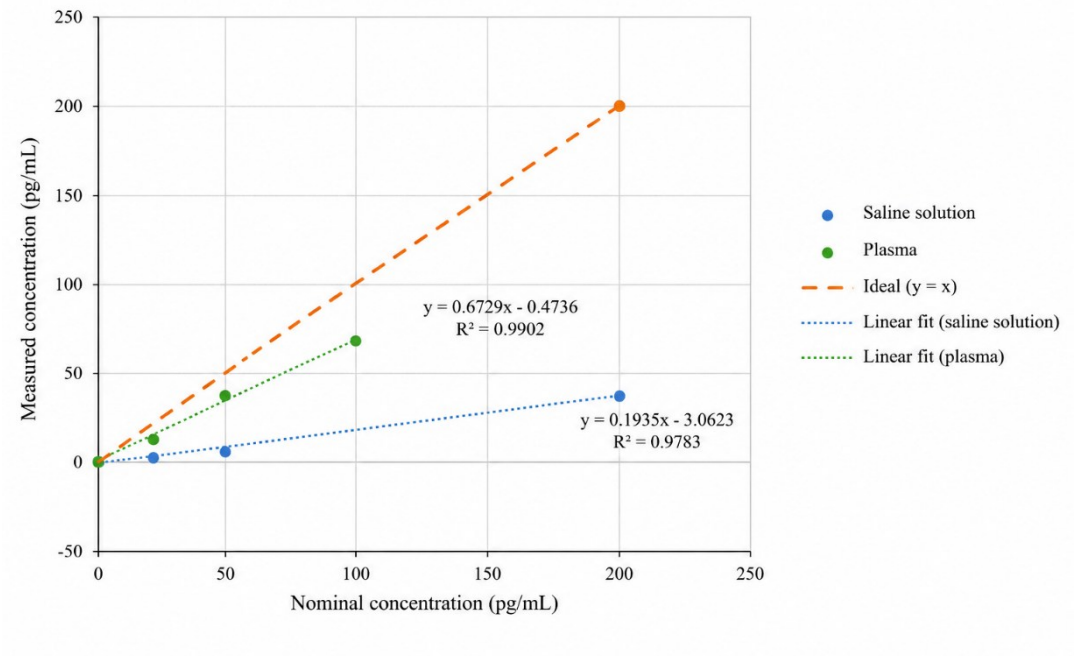


Figure 6:Comparison between nominal and measured IL-6 concentrations obtained with the Finecare™ FIA Meter Plus in Standard mode for saline solution and spiked plasma samples. The dashed line represents the ideal response (y = x), while the dotted lines represent the linear regressions obtained for the two matrices, with the corresponding equations and coefficients of determination (R^2).

2.5 Considerations on Usability, Cost and Performance

Following the experimental characterisation described in the previous section, an overall assessment of the Finecare™ FIA Meter Plus can be made by considering not only the observed analytical performance, but also usability, testing time and the costs associated with the system. These aspects are particularly relevant in the evaluation of a device. The advantages of a POC over conventional laboratory methods depend on both the accuracy and the speed with which it provides results using simple procedures.

Finecare™ FIA Meter Plus has several features that would make it suitable for Point-of-Care applications. The system combines the analytical reader and an integrated printer within a single instrument, allowing results to be immediately documented [37]. The maximum sample volume required by the IL-6 assay is 75 µL [38].

The use of relatively small sample volumes is advantageous, particularly when only limited amounts of biological material are available. Furthermore, analyte-specific kits allow different measurements to be performed using the same analyser, without requiring separate instrumentation for each biomarker [37].
The operating procedure is relatively straightforward and standardised. Once the sample has been prepared, the operator selects the appropriate matrix type, dispenses the required volume according to the assay protocol and performs the measurement using the selected operating mode. Automatic reporting of a quantitative result also avoids the visual interpretation required by some qualitative or semi-quantitative rapid tests [37,38].
Another advantage is the possibility of analysing different biological matrices. The Finecare™ platform supports assays performed on whole blood, serum/plasma, urine, or other assay-specific biological samples, depending on the specific test kit [37]. In particular, the IL-6 assay used in the present study is intended for the quantitative determination of IL-6 in human whole blood, serum, or plasma [38]. This versatility is particularly relevant in a POC setting, where limiting sample pretreatment steps may contribute to reducing the overall time required to obtain a result.
A particularly relevant feature of the Finecare™ system is the availability of two operating modes, Standard and Quick [37,38]. Comparison of the experimental results showed that the two modes generally yielded values of the same order of magnitude. In samples prepared in saline solution, the mean Quick measurements deviated only slightly from the corresponding Standard measurements, with absolute differences, calculated as mean Quick value minus Standard value, ranging from 0.0 pg/mL at the nominal concentration of 50 pg/mL to +2.0 pg/mL at 200 pg/mL.
A similar pattern was observed in plasma samples. The mean Quick measurements showed absolute differences of 0.0, +1.17 and +3.9 pg/mL compared with the corresponding Standard measurements at nominal IL-6 concentrations of 20, 50 and 100 pg/mL, respectively. Thus, the difference between the two operating modes increased slightly with concentration, with Quick mode tending to yield higher values at the higher concentration levels.
Overall, no differences were observed that would suggest substantially different behaviour between the two operating modes. At the higher concentrations, however, Quick determinations tended to yield slightly higher values than the corresponding Standard measurements. Therefore, the marked underestimation observed during the experiments does not appear to be attributable solely to the selected measurement mode, since it was present in both Standard and Quick determinations.
Regarding the three consecutive Quick measurements, the low data dispersion was highly significant. As previously outlined, the plasma sample coefficients of variation never exceeded 2.5% across all tested concentrations. This outcome clearly demonstrates the divergence between accuracy and repeatability. Despite exhibiting a systematic deviation from nominal values, the system consistently generated highly reproducible results across sequential trials.
The possibility of obtaining results more rapidly while maintaining limited variability among consecutive measurements represents a favourable feature in a

Point-of-Care setting. Nevertheless, the limited number of samples and replicates included in the present study does not allow formal analytical equivalence between the two operating modes to be established. A conclusive assessment would require additional testing on a larger number of samples across the full analytical measurement range of the assay.

The experiment found a substantial difference between the two matrices. In plasma, although systematic underestimation was present, the mean recovery for standard measurements was approximately 63%, while in saline it was approximately 8%. From this variation in results we can deduce the strong dependence on the matrix used.

This result is particularly relevant when considering the operating principle of the test. The Finecare™ IL-6 test is a fluorescence immunoassay intended for the quantitative determination of IL-6 in human whole blood, serum, or plasma [38]. Sample composition can influence several processes involved in the analytical procedure, including antigen–antibody interactions, sample transport through the reaction system and the overall efficiency of immunocomplex formation and detection. Consequently, the use of a simplified matrix such as saline solution, which is not among the matrices specified for the IL-6 assay, may not adequately reproduce the physicochemical conditions for which the system was developed and calibrated. This factor may contribute to the marked reduction in recovery observed in samples prepared in saline solution.

It is important, however, to emphasise that the results obtained do not allow the observed underestimation to be attributed to a single cause. In addition to matrix effects, other experimental factors may have contributed to the observed underestimation. In particular, the stability of IL-6 may have been affected by the experimental conditions used for sample preparation, dilution, storage and handling, potentially reducing the amount of analyte available for detection. However, IL-6 stability was not specifically investigated in the present experiments and this possibility should therefore be regarded only as a hypothesis.

Determining the contribution of each individual factor would require additional experiments specifically designed to distinguish their respective effects.

A further observation from the preliminary experiments concerns system performance at low concentrations. Samples spiked to expected concentrations of 6.25 and 8.88 pg/mL yielded values close to or below 3 pg/mL, whereas increasing the expected concentration to 24.4 pg/mL resulted in measurements substantially closer to the nominal value. This observation further supports the need for caution when interpreting measurements in the lower region of the analytical range.

Overall, the experiments therefore highlight two distinct aspects of system performance. On one hand, consecutive determinations showed good repeatability, as indicated by the limited variability of Quick measurements; on the other hand, systematic underestimation of the nominal concentration was observed, particularly in samples prepared in saline solution and, to a lesser extent, in plasma samples.

In addition to analytical and operational performance, cost is a key parameter when assessing the applicability of a Point-of-Care technology. For the Finecare™ FIA Meter Plus, an economic assessment can be based directly on the commercial quotation received for acquisition of the system.

The list price of the Finecare™ FIA Meter Plus analyser reported in the quotation was €3,750.00.
The analyser was purchased together with assay kits for the biomarkers of interest, with an average price of approximately €235 for 25 tests.
For the specific assay used in the present study, the Finecare™ IL-6 kit was priced at €325 for 25 determinations, corresponding to a theoretical reagent cost of approximately €13 per test.
This represents only the direct cost of the assay kit and does not include additional costs related to personnel, supplementary consumables, quality control and maintenance.
When compared with the alternative POC systems considered during the preliminary market survey, the Finecare™ platform was also competitive from an economic perspective. The commercial quotations collected during the device-selection phase showed differences in both analyser purchase price and assay costs among the evaluated platforms. Finecare™ therefore represented a favourable compromise between initial investment, cost per determination, availability of the biomarkers of interest and the technical and operational characteristics required for the present study.
Considering the initial investment alone, the cost of the analyser appears relatively modest compared with that typically associated with centralised analytical instrumentation. However, a comprehensive economic assessment would require comparison with alternative methods, taking into account not only the purchase price but also sample throughput, cost per determination, operator time, maintenance requirements and overall turnaround time.
When operational, economic and analytical aspects are considered together, the Finecare™ FIA Meter Plus offers several features that may favour its use as a Point-of-Care platform. These include the relative simplicity of the procedure, the small sample volume required, the availability of quantitative assays for different biomarkers and the presence of a Quick mode designed to reduce analysis time [37,38]. The initial instrument cost and per-test cost may also represent favourable features for a platform intended for decentralised testing.
The experimental results nevertheless highlighted several aspects that require caution. In particular, the underestimation of nominal IL-6 concentrations indicates that system performance may be influenced by the experimental conditions and the matrix used. The marked difference observed between plasma and saline solution therefore emphasises the importance of performing measurements using matrices intended for the specific assay [38].
Overall, the Finecare™ system can be regarded as a versatile and operationally straightforward POC platform, with potential advantages related to rapid analysis and the ability to perform different assays on the same analyser [37]. However, the results obtained in the present work demonstrate that the assessment of a Point-of-Care system cannot be based solely on speed and ease of use, but must also include careful characterisation of analytical performance under the actual conditions of use. In particular, for IL-6 determination, further studies involving a larger number of biological samples and comparison with a reference method would be required to define system performance and reliability in clinical applications more accurately.

An important consideration arising from the present assessment is therefore the trade-off between speed, operational simplicity, cost and analytical quality, which represents one of the main factors to be considered when selecting and implementing a Point-of-Care technology.

Chapter 3

Experimental Study of OECT Electrochemical Devices

3.1 Introduction to OECT Technology and Its Applications

Organic Electrochemical Transistors (OECTs) are a class of organic transistors that have attracted increasing interest in recent years in the fields of bioelectronics and biosensing. One of the main features that makes these devices particularly suitable for biomedical applications is their ability to interface ionic signals, which are characteristic of biological systems, with electronic signals that can be readily acquired and processed; in addition, the organic materials used for their fabrication offer advantageous properties such as low-temperature processability, compatibility with flexible substrates, large-area fabrication and adaptability to different device architectures [13].

From a structural point of view, an OECT consists of three main electrodes, referred to as source, drain and gate. The source and drain are connected through a channel made of an organic semiconducting or conducting material, while the gate is coupled to the channel through an electrolyte, which plays a fundamental role in device operation by enabling the movement of ionic species between the gate and the organic material forming the channel [13].

In OECTs the channel material is permeable to ions and the application of a gate voltage, indicated as V_{GS}, induces ion migration from the electrolyte into the channel,

or vice versa, thereby modifying the doping state of the organic material and consequently its electrical conductivity. Since this process takes place throughout the volume of the active material rather than being restricted to its surface, OECTs are characterised by a volumetric capacitance [13].

One of the most widely used materials for OECT channels is poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate), commonly referred to as PEDOT:PSS, which combines high electrical conductivity with good stability in aqueous environments and has therefore been extensively employed in bioelectronic applications. In PEDOT:PSS-based devices, the channel is already conductive in the absence of a positive gate voltage; when cations from the electrolyte enter the PEDOT:PSS film, they progressively compensate the negative charges of PSS and induce the reduction of oxidised PEDOT, thereby decreasing the concentration of mobile charge carriers within the channel and, consequently, reducing the source-drain current (I_{DS}). For this reason, PEDOT:PSS-based OECTs are generally described as operating in depletion mode [13].

The current flowing through the channel depends on both the voltage applied between source and drain (V_{DS}) and the voltage applied between gate and source (V_{GS}) and the electrical behaviour of the device can therefore be characterised using different types of curves. The output characteristics describe the variation of I_{DS}as a function of $V_{DS}$for different values of V_{GS}, whereas the transfer characteristics describe the variation of I_{DS}as a function of V_{GS}, generally while keeping V_{DS}constant; the latter are particularly relevant in biosensing applications because they make it possible to evaluate changes in the electrical properties of the transistor resulting from modifications occurring at the gate-electrolyte interface [13–15].

A particularly relevant parameter in OECT characterisation is the transconductance (g_m), defined as the variation in drain current with respect to a variation in gate voltage:

$$g_m = \frac{\partial I_{DS}}{\partial V_{GS}}$$

Transconductance therefore expresses the ability of the gate to modulate the current flowing through the channel and a high $g_m$means that a small variation in the applied gate potential, or in the effective potential experienced by the gate, can produce a relatively large variation in the drain current. This property represents one of the main advantages of OECTs for sensing applications, since the transistor does not simply act as a transducer but can also provide intrinsic amplification of the signal [13,42].

The mechanism through which a biological event is converted into an electrical variation depends on the architecture and operating mode of the device. In OECT-based biosensors, the gate can play a central role in the recognition process because its surface can be modified with molecules capable of selectively recognising a specific analyte, including antibodies, aptamers, enzymes, or other biorecognition

elements; the interaction between the analyte present in the sample and the immobilised bioreceptor can alter the properties of the gate-electrolyte interface and, consequently, the effective electrochemical modulation of the channel. These changes can then be detected through variations in I_{DS}, shifts or modifications in the transfer characteristics, changes in transconductance, or other electrical parameters associated with transistor operation [13,14,15].
This configuration makes it possible, at least to some extent, to separate the surface responsible for biological recognition from the channel responsible for charge transport and signal amplification. Gate functionalisation therefore represents a particularly attractive strategy for biosensing applications, since the specificity of the sensor can be modified by acting directly on the recognition surface without necessarily altering the material or structure of the transistor channel.
Interest in OECTs for biomedical applications also arises from their ability to operate directly in aqueous and electrolytic environments, a condition particularly well suited to the analysis of biological samples. The combination of ionic–electronic transduction, low-voltage operation and signal amplification has supported the use of these devices in several areas of bioelectronics, including physiological signal monitoring, interfaces with cells and tissues and the development of biosensors for the detection of different analytes [13,42].
In recent years, particular attention has been devoted to the use of OECTs for the detection of proteins and biomarkers associated with pathological processes, since the possibility of functionalising the gate with specific recognition elements allows the same basic device architecture to be adapted to different molecular targets.
An antibody-based gate functionalisation strategy was also investigated by Preziosi et al. for the detection of transglutaminase 2 (TG2). The authors developed an OECT immunosensor in which changes in the electrical response of the device, particularly in parameters related to transconductance, were used to investigate the recognition of the target protein at very low concentrations [14]. This study provides an example of how antibody-based molecular recognition at the gate can be coupled with the intrinsic amplification properties of OECTs for protein sensing.
An immunorecognition-based approach has also been used for the detection of the SARS-CoV-2 Spike protein. In this case, analysis of the electrical characteristics of the transistor showed that changes in parameters related to transconductance could be correlated with protein concentration. The study also emphasised the importance of surface functionalisation in converting the antigen–antibody interaction into a measurable change in the electrical response of the transistor [15].
The versatility of OECTs has also been demonstrated using recognition strategies other than antibodies. For example, Qian et al. developed an OECT for the detection of C-reactive protein (CRP) using a PEDOT gate functionalised with phosphorylcholine groups, an approach designed to combine recognition capability with antifouling properties. This aspect is particularly important when measurements are performed in complex biological matrices, where non-specific protein adsorption may affect the selectivity and overall performance of the biosensor [43].

Functionalisation strategies reported in the literature are diverse and depend on both the nature of the target and the material used for the gate electrode [13]. In addition to the more common immobilisation of antibodies, approaches based on different recognition elements, including aptamers and nanobodies, as well as functional surfaces designed to reduce non-specific adsorption, have also been developed [42–44]. In the case of gold electrodes, a frequently used strategy involves the formation of self-assembled monolayers containing functional groups that can be used for the subsequent immobilisation of the bioreceptor [13]. The choice of surface chemistry therefore represents an important aspect in biosensor design, as it directly affects the characteristics of the biofunctional interface and, consequently, the efficiency and selectivity of molecular recognition [13,43].

OECT architectures based on aptamers have also been proposed. Aptamers are oligonucleotide sequences selected for their ability to bind specifically to a target molecule and, compared with antibodies, offer several interesting characteristics in terms of synthesis, chemical modification and stability. They can also be immobilised on the gate surface through different functionalisation strategies.

Diacci et al. applied this approach to the detection of interleukin-6 (IL-6), developing an OECT with a PEDOT:PSS gate modified with gold nanoparticles and subsequently functionalised with an IL-6-specific aptamer. The study reported detection of the analyte over a concentration range extending from the picomolar to the nanomolar level and evaluated the selectivity of the system against other cytokines involved in inflammatory processes [44]. This work is particularly relevant to the present thesis, as it demonstrates the possibility, at least under experimental conditions, of using a functionalised OECT to convert IL-6 recognition into a measurable change in the electrical properties of the transistor.

IL-6 is indeed a particularly interesting target for the development of biosensors, as it is a cytokine involved in the regulation of immune and inflammatory responses and its concentration can vary significantly under different pathological conditions. However, cytokine detection presents important analytical challenges, since these molecules may be present at very low concentrations in biological fluids. Although reference methods provide high sensitivity and specificity, they may require dedicated instrumentation, relatively complex procedures and analysis times that are not always compatible with Point-of-Care applications. The development of platforms capable of combining molecular recognition, direct transduction and signal amplification therefore represents an area of considerable interest [13,44].

Several studies have also shown that the high amplification capability of OECTs can be exploited for protein detection at extremely low concentrations. Guo et al., for example, developed nanobody-functionalised OECTs for the detection of viral antigens in complex biological fluids, showing how volumetric coupling between ionic and electronic charges within the channel can contribute to the amplification of binding events occurring at the gate [42]. These findings illustrate the potential of OECT technology for applications requiring high sensitivity and small sample volumes.

However, the performance achieved under controlled experimental conditions does not necessarily imply that the technology can be easily transferred to clinical practice. The development of an OECT-based biosensor requires the simultaneous control of several factors, including the properties of the organic material, device geometry, electrolyte stability, gate characteristics, density and orientation of the immobilised bioreceptors, non-specific adsorption and stability of the electrical measurements. The final response of the device therefore results from the interplay of electronic, ionic, electrochemical and biological phenomena, making its optimisation more complex than that of a simple molecular recognition system [13].
For future Point-of-Care applications, additional aspects such as device-to-device reproducibility, long-term stability, operation in real biological matrices, simplicity of the measurement protocol and the possibility of large-scale fabrication are also essential. The literature therefore presents OECTs as highly promising biosensing platforms, while at the same time showing that the transition from experimental prototypes to robust diagnostic systems still requires careful optimisation and validation [13].
Based on these characteristics, OECTs were selected in the present thesis as an experimental platform to complement the evaluation of the commercial POC system described in Chapter 2. In particular, the experimental work focused on investigating the possibility of using a functionalised OECT for IL-6 recognition.

3.2 Materials and Methods

3.2.1 Functionalization and Preparation of the Devices

Device preparation and functionalization represented a key step of the experimental work since, in the OECT architecture adopted in this study, analyte recognition occurs at the gate electrode. As discussed in the previous section, functionalization of the gate surface makes it possible to introduce a specific recognition element without directly modifying the organic channel responsible for charge transport and signal modulation [13,14,15]. In this work, the aim of the functionalization procedure was therefore to obtain a surface capable of selectively recognizing interleukin-6 (IL-6) through the immobilization of an anti-IL-6 antibody.
The procedure adopted consisted of four main steps: formation of a self-assembled monolayer (SAM) using 3-mercaptopropionic acid (3-MPA), activation of the carboxyl groups using EDC/Sulfo-NHS chemistry, immobilization of the anti-IL-6 antibody and subsequent treatment with ethanolamine.
In the system investigated in this work, functionalization was performed on silver gate electrodes. The first step involved the formation of the SAM using 3-MPA, a bifunctional molecule containing a thiol group and a terminal carboxyl group. The purpose of using this molecule was to chemically modify the electrode surface by introducing carboxyl groups available for subsequent activation and conjugation reactions with the bioreceptor.

The functionalization protocol also included a preliminary cleaning step using acetone and 2-propanol, aimed at removing possible contaminants from the electrode surface before SAM formation.
For SAM formation, the electrodes were incubated in a solution containing 10 mM 3-MPA in ethanol for 1 h at room temperature under gentle agitation. At the end of the treatment, the samples were washed twice with fresh ethanol and once with ultrapure water in order to remove material that was not stably associated with the surface.
Following monolayer formation, the carboxyl groups present on the surface were activated. Before activation, the electrodes were equilibrated for 15 min at room temperature in MES buffer at pH 4.7, consisting of 0.1 M 2-(N-morpholino)ethanesulfonic acid containing 0.9% NaCl. The electrodes were then incubated for an additional 15 min at room temperature in a solution containing 10 mM EDC and Sulfo-NHS, prepared in the same MES buffer. At the end of this step, excess reagents were removed by three consecutive washes in PBS, each lasting 5 min.
EDC/Sulfo-NHS chemistry was used to activate the carboxyl groups introduced during the previous surface modification and make them available for subsequent conjugation with the antibody. EDC, or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, acts as a carbodiimide coupling reagent, whereas Sulfo-NHS (N-hydroxysulfosuccinimide) promotes the formation of a more stable amine-reactive intermediate. The subsequent reaction with primary amino groups present in the antibody enables the formation of covalent amide bonds and therefore the immobilization of the bioreceptor on the modified surface.
The next step involved the conjugation of the anti-IL-6 antibody, which was used as the specific recognition element. The previously modified electrode was incubated overnight at 4 °C with 80 µL of an anti-IL-6 antibody solution at a concentration of 5 µg/mL in PBS, with the sample maintained in a humid chamber.
The prolonged incubation at controlled temperature was intended to promote contact between the activated surface and the antibody, allowing its immobilization before the subsequent washing steps. At the end of the incubation, the electrodes were washed twice for 5 min with PBS containing 0.05% (v/v) Tween 20 under gentle agitation, followed by an additional wash with PBS.
The washing step following conjugation was intended to remove molecules that were not stably associated with the surface. The presence of Tween 20 in the washing buffer was also intended to limit non-specific interactions. In this way, the resulting surface was designed to present immobilized anti-IL-6 antibodies available for subsequent interaction with the target analyte.
After antibody immobilization, the procedure included an additional surface treatment with 1 M ethanolamine in PBS for 1 h at room temperature. This step was intended to block residual activated groups that remained available after antibody conjugation, thereby reducing the number of reactive sites potentially involved in non-specific interactions.

This aspect is particularly relevant in a biosensing system, since non-specific interactions between the gate surface and species present in the solution could contribute to variations in the electrical signal, making it more difficult to attribute such changes specifically to IL-6 recognition.
Overall, the functionalization procedure was designed to obtain a recognition interface capable of binding IL-6 through antigen–antibody interaction and to subsequently determine whether this binding event could result in a measurable change in the electrical response of the OECT. Antibody-functionalized gate configurations have previously been employed in OECT-based protein sensing, demonstrating the possibility of coupling selective molecular recognition at the gate with modulation of the transistor response [14,15].
The preparation of the recognition interface provided the common basis for the subsequent experimental tests, while the specific operating conditions, solution compositions and modifications adopted during the different measurement campaigns are described in the following section.

3.2.2 Measurement Protocols

The experimental activity aimed at evaluating the response of the OECT to the presence of IL-6 was organized into four successive measurement campaigns. The campaigns were designed progressively, maintaining the functionalization strategy described in Section 3.2.1 as a common basis while modifying selected experimental conditions in successive trials. In particular, different compositions of the measurement solutions and different IL-6 concentrations were considered, with the aim of identifying conditions capable of providing a sufficiently stable electrical response and verifying the possible occurrence of variations associated with the interaction between IL-6 and the antibody immobilized on the gate.
In each campaign, a gate electrode functionalized with the anti-IL-6 antibody was accompanied by a non-functionalized electrode used as a control. During the antibody conjugation step, the sensing electrode was incubated with the anti-IL-6 antibody solution, whereas the control electrode was maintained in PBS under the same experimental conditions. The control was included to assess whether variations observed during exposure to IL-6-containing solutions could be associated with the presence of the immobilized bioreceptor rather than with the intrinsic electrical behaviour of the system or the measurement conditions.
In the first campaign, electrode preparation was carried out according to the procedure described in Section 3.2.1. During SAM formation, the electrodes were incubated in 10 mM 3-MPA in ethanol for 1 h at room temperature under gentle agitation, with the surface to be functionalized facing the bottom of the container. After two washes with fresh ethanol and one wash with ultrapure water, the carboxyl groups were activated using EDC/Sulfo-NHS in MES buffer. The sensing electrode was subsequently incubated overnight with 80 µL of anti-IL-6 antibody solution at a concentration of 5 µg/mL in PBS, whereas the control electrode was incubated with PBS alone. Following the post-conjugation washes, the

functionalized gate was treated with 1 M ethanolamine in PBS for 1 h at room temperature to block residual activated groups.
For this first campaign, two solutions were prepared for the measurements: PBS 1×, used as the reference solution and a solution containing IL-6 at a final concentration of 2 pM together with 75 nM BSA in PBS.
The solution containing IL-6 was prepared from the lyophilized protein. This was initially resuspended in PBS and thus a primary solution was obtained. Serial dilutions in PBS containing BSA were then performed. Finally, a final IL-6 concentration of 2 pM was obtained, corresponding to approximately 47.4 pg/mL. BSA was added to the solution to improve protein stability during measurement. The detailed preparation procedure is given in Appendix 1.
The availability of an IL-6-free reference solution and an IL-6-containing solution allowed the device to be sequentially exposed to the two experimental conditions. During measurement, the solutions were flowed over the system in order to evaluate the OECT response during the transition from the reference solution to the IL-6-containing solution. The primary objective of this first campaign was to preliminarily assess whether exposure of the functionalized gate to the target analyte was accompanied by a recognizable variation in the electrical response relative to the initial condition.
The observations obtained during the first campaign were useful in defining the conditions to be used during subsequent tests. Duarnete the second campaign used the same functionalization and measurement approach used in the first campaign. The modifications consist exclusively of the preparation of the electrodes and the composition of the measurement solutions.
The main change to the preparation procedure was the addition of a drying step under nitrogen flow after post-conjugation washes. All preceding steps, including SAM formation, EDC/Sulfo-NHS activation, overnight antibody conjugation and washing, were performed as described for the first campaign. After nitrogen drying, the functionalized gate was treated with 1 M ethanolamine in PBS for 1 h at room temperature.
For this campaign, three measurement solutions were prepared: a solution containing 10.5 nM IL-6 and 375 nM BSA in PBS, a PBS-only reference solution and PBS containing 0.1% BSA without IL-6. Compared with the first campaign, the IL-6 concentration was therefore substantially increased. In addition, a BSA-containing solution without IL-6 was introduced to evaluate whether the presence of the stabilizing protein itself could contribute to the measured response independently of the target analyte. The detailed preparation of the solutions is reported in Appendix 2.
For consistency with the concentration reported in molar units, 375 nM BSA corresponds approximately to 0.0025% (w/v), assuming a BSA molecular mass of approximately 66.5 kDa.
The third campaign was based on the procedure adopted in the second campaign, with an additional modification to electrode preparation. Specifically, after SAM formation and the subsequent ethanol and ultrapure-water washes, the electrodes

were dried under nitrogen flow before activation of the carboxyl groups. The remaining activation, antibody conjugation, washing and ethanolamine treatment steps were performed as in the second campaign, including nitrogen drying after the post-conjugation washes.
The three-solution configuration introduced in the second campaign was maintained. The IL-6-containing solution consisted of 10.5 nM IL-6 together with 375 nM BSA, whereas the reference solutions consisted of 0.01× PBS and 0.01× PBS containing 0.1% BSA. Thus, the IL-6 and BSA concentrations in the analyte-containing solution were maintained, while the concentration of the reference buffer was reduced from 1× PBS to 0.01× PBS. Detailed solution preparation is reported in Appendix 3.
The fourth campaign maintained the general preparation procedure described above but introduced a further modification following antibody conjugation. After the post-conjugation washes, the electrodes were dried under nitrogen flow; however, the subsequent ethanolamine treatment was omitted in this campaign.
The measurement protocol was also simplified with respect to the second and third campaigns. Two types of solution were used: 0.01× PBS, serving as the reference solution and IL-6-containing solutions prepared in PBS. BSA was not included in the measurement solutions in this campaign. Two IL-6 concentrations, 10.5 nM and 2.1 nM, were investigated. The detailed preparation procedure is reported in Appendix 4.
Overall, the four measurement campaigns represented a progressive modification of the experimental conditions adopted to investigate the response of the OECT system to IL-6. The IL-6 concentration was changed from 2 pM in the first campaign to 10.5 nM in the second and third campaigns, while two concentrations, 10.5 nM and 2.1 nM, were evaluated in the fourth campaign. In parallel, the composition of the reference and measurement solutions was modified through the introduction of BSA-containing controls, the reduction of the PBS concentration from 1× to 0.01× and the subsequent removal of BSA from the measurement solutions in the fourth campaign.
The functionalization protocol was also progressively modified. Nitrogen-drying steps were introduced during the second and third campaigns, while ethanolamine treatment was omitted in the fourth campaign. These modifications were introduced on the basis of the observations obtained during the successive experimental measurements, which are presented and discussed in the following sections.

3.3 Results

For each campaign, transfer curves (drain current as a function of gate voltage) were acquired both on the electrode functionalized with anti-IL-6 antibody and on the non-functionalized control electrode, replicating the measurement across the three available devices (dev1, dev2, dev3). For each device, in addition to the

measurement in the presence of the IL-6-containing solution, two further curves were acquired under two different measurement-circuit configurations, with and without short-circuiting, in order to verify whether the shape of the transfer curve was influenced by the electrical configuration of the system rather than by the solution in contact with the gate.
During the measurements, the different solutions were delivered to the gate electrode through a microfluidic system connected to a syringe pump. The syringe pump provided a controlled and continuous flow of the selected solution through the microfluidic circuit and over the gate surface. This configuration made it possible to sequentially expose the same gate electrode to the different solutions considered in each experimental campaign without modifying the position of the device. When the measurement solution was changed, the syringe pump was used to introduce the subsequent solution through the same fluidic path, allowing the electrical response of the OECT to be monitored under the different experimental conditions.
In the first campaign, on the functionalized gate, the curves acquired across the three devices, under the different configurations, largely overlap with one another, without a clear separation attributable either to the solution used during the measurement (reference PBS solution or IL-6-containing solution) or to the circuit configuration (with or without short circuit). A minimal dispersion between the curves is visible in all three devices, more pronounced for dev2 and dev3, but of a magnitude comparable to that observed on the control electrode, described below.
Similarly, on the non-functionalized gate (control electrode), the curves acquired across the three devices, under the same measurement configurations, show the same behaviour: a good degree of overall overlap, with a dispersion in drain current amplitude comparable to that observed on the functionalized electrode. This result indicates that the variations observed between the different curves are not specifically associated with the presence of the antibody, the target, or the electrical configuration of the circuit, but are rather compatible with the intrinsic variability of repeated measurements.
The time evolution of the drain current during prolonged exposure of the device to a solution was also recorded (dev1 vs Time). The trace shows an initial settling phase of the current, followed by a step increase of significant amplitude, after which the signal stabilizes at a new, essentially constant level. Since this step variation is not followed by the slow decay expected in the case of specific antigen–antibody binding, but rather by a stable plateau, it was not considered a significant response associable with IL-6 recognition, but more plausibly an instrumental artefact or a change in the measurement conditions.
Overall, the results of the first campaign did not reveal an electrical response clearly and specifically attributable to the interaction between IL-6 and the antibody immobilized on the gate.

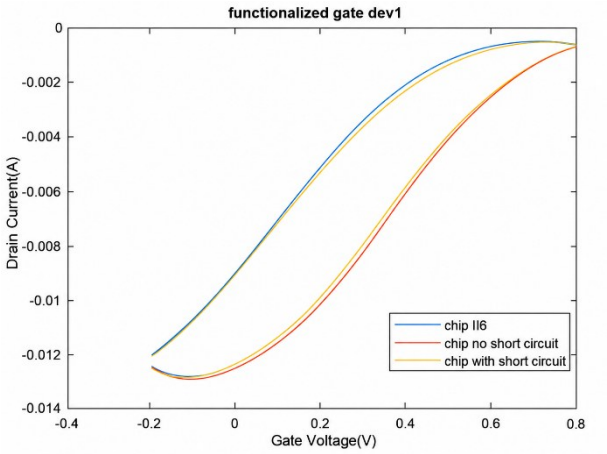


Figure 7: Transfer curves acquired on device dev1 using the anti-IL-6-functionalized gate under the different measurement conditions investigated during the first experimental campaign.

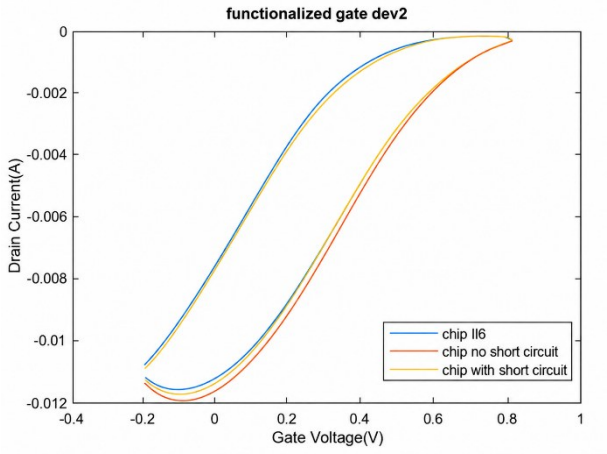
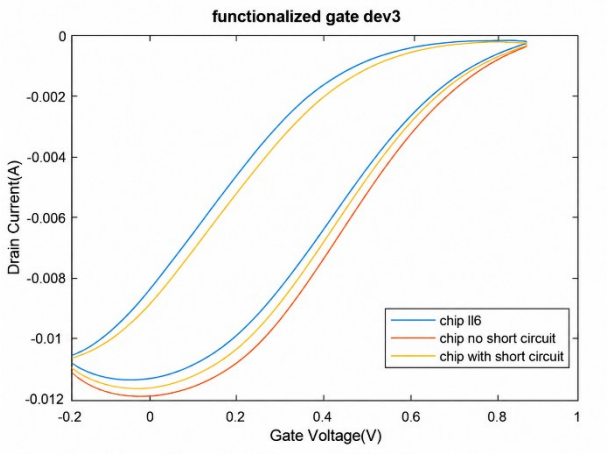


Figure 8: Transfer curves acquired on device dev2 using the anti-IL-6-functionalized gate under the different measurement conditions investigated during the first experimental campaign.



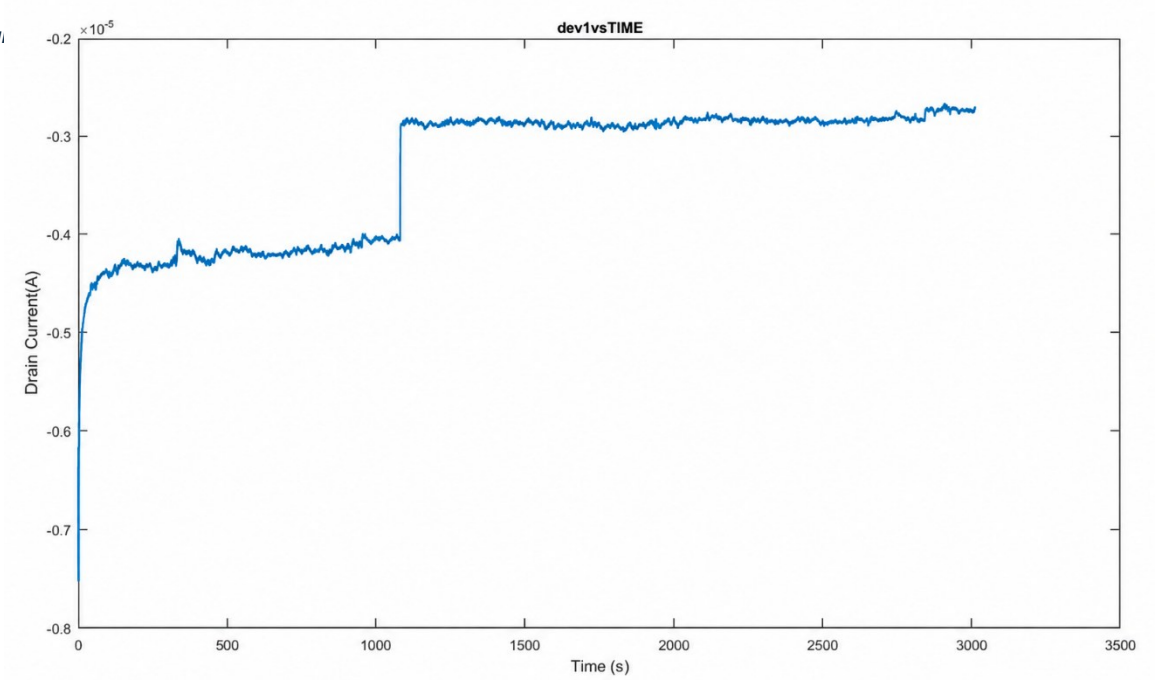


Figure 10: Time evolution of the drain current recorded for device dev1 during the first experimental campaign, showing an initial settling phase followed by a step increase and subsequent signal stabilization.

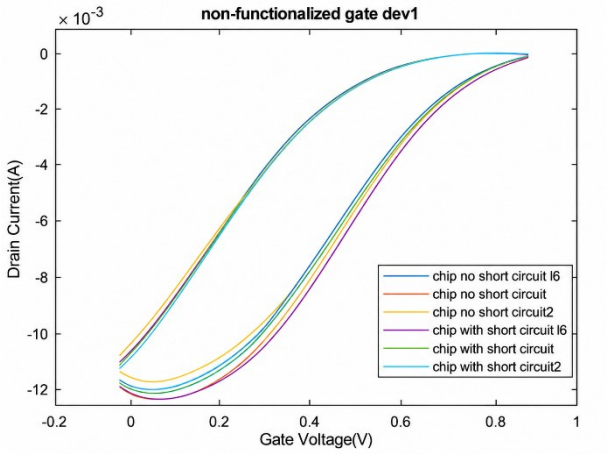


Figure 11: Transfer curves acquired on device dev1 using the non-functionalized control gate under the different measurement conditions investigated during the first experimental campaign.

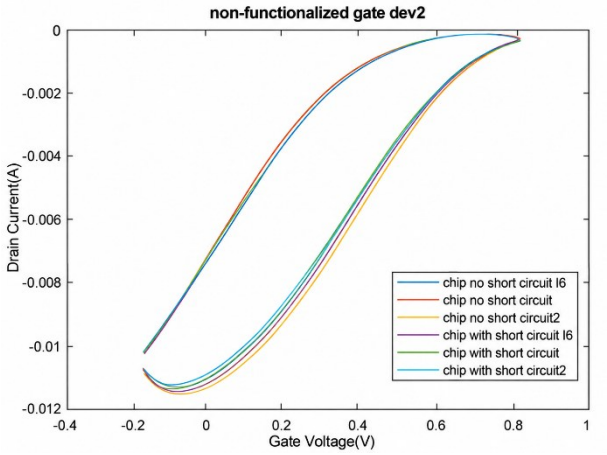
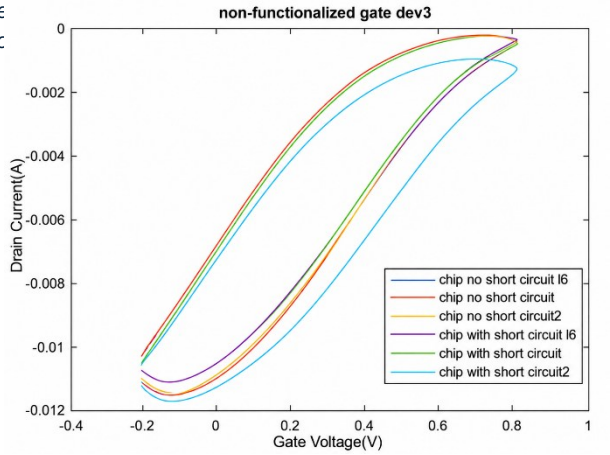


Figure 12: Transfer curves acquire 

under the different measurement cc n.

Figure 13: Transfer curves acquired on device dev3 using the non-functionalized control gate under the different measurement conditions investigated during the first experimental campaign.

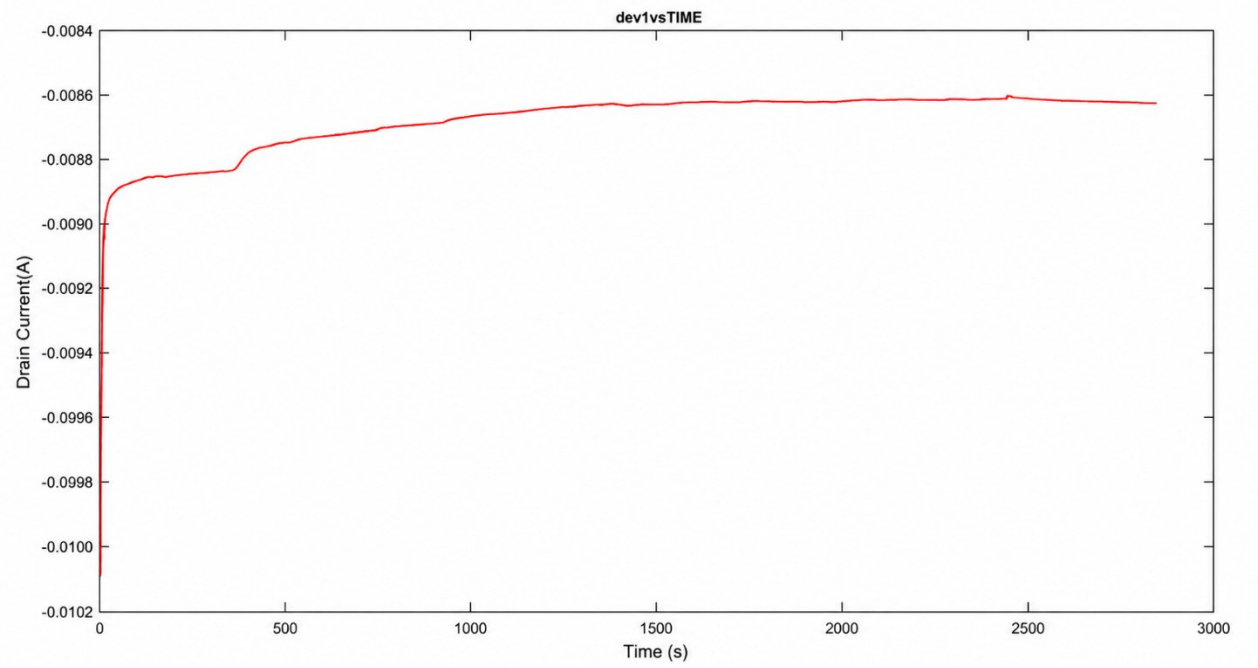


Figure 14: Time evolution of the drain current recorded for device dev1 during the experimental measurement, showing an initial rapid variation followed by a gradual stabilization of the signal.

Due to a malfunction of device dev1, the transfer curves for the second campaign were recorded exclusively on devices dev2 and dev3, exposing the functionalized

gate in sequence to the different solutions specified by the protocol (PBS, PBS + BSA + IL-6).
On both devices, the curves acquired under the different measurement conditions overlap with one another, without a systematic separation attributable to the solution in contact with the gate at the time of acquisition. For both devices the curves obtained in the presence of IL-6 are not recognizably distinguishable from those acquired with the reference solution alone.
This lack of a clear separation between conditions represents a critical result for the validation of the system. The overlap observed therefore indicates that, also in this second campaign, the system was not able to provide a discriminating response with respect to the presence of the analyte.

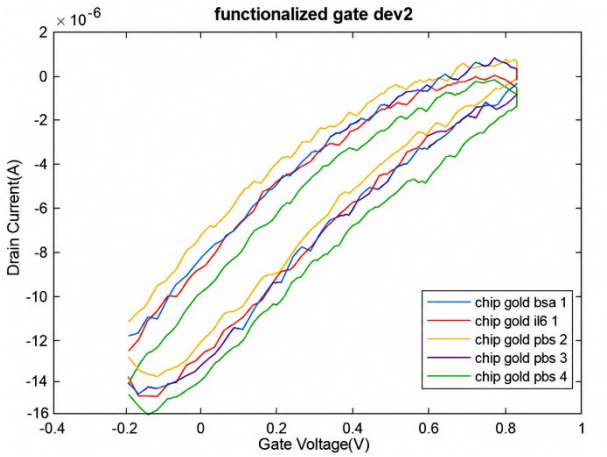


Figure 15: Transfer curves acquired on device dev2 using the anti-IL-6-functionalized gate during the second experimental campaign, under the different measurement conditions involving PBS, BSA and IL-6-containing solutions.

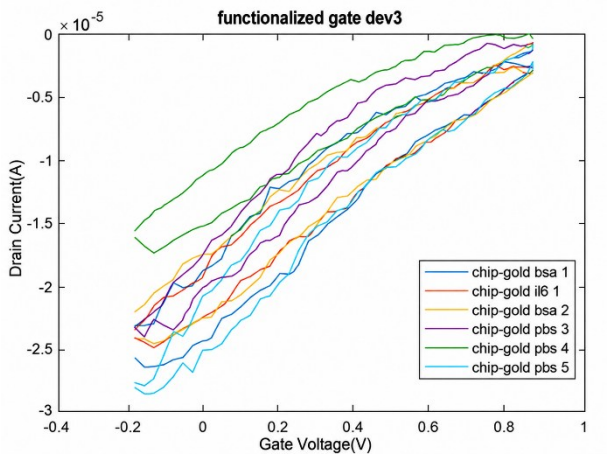


Figure 16: Transfer curves acquired on device dev3 using the anti-IL-6-functionalized gate during the second experimental campaign, under the different measurement conditions involving PBS, BSA and IL-6-containing solutions.

For this campaign, the transfer curves (drainage current as a function of gate voltage) were

acquired on the three devices used (dev1, dev2, dev3). On the
functionalized gate was exposed to PBS 0.01× solution and PBS + BSA + IL-6 solution. For
reasons for experimental timing, measurement with PBS + BSA alone (solution in the absence of IL-6) was not performed.
For device dev1, the acquired curves show a visible dispersion in terms of drain current amplitude between the different PBS repetitions and the measurement in the presence of IL-6, without, however, a clear and systematic separation univocally attributable to the type of solution used. For device dev2, the acquired curves show a more gradual trend, with a progression of current amplitudes across the different repetitions, likewise not clearly attributable to the composition of the solution. For device dev3, finally, the curve obtained with the PBS + BSA + IL-6 solution stands out from the PBS repetitions by a slightly higher current amplitude, while the repetitions with the reference solution are superimposable on one another.
Overall, also in this third campaign, the variations observed between the different measurement conditions did not allow a clear identification of an electrical response specifically attributable to the presence of IL-6.

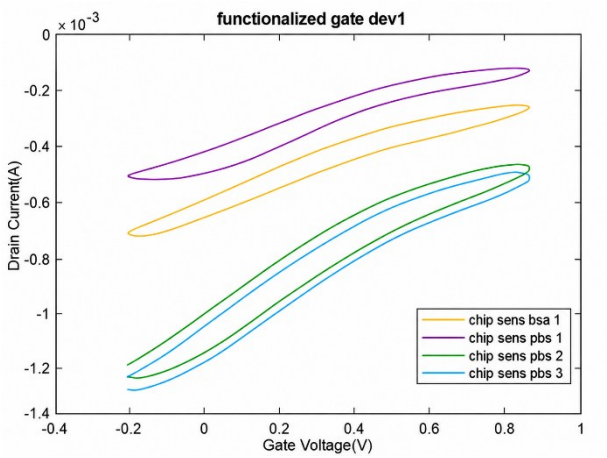


Figure 17: Transfer curves acquired on device dev1 using the anti-IL-6-functionalized gate during the third experimental campaign, under the different measurement conditions involving PBS and BSA-containing solutions.

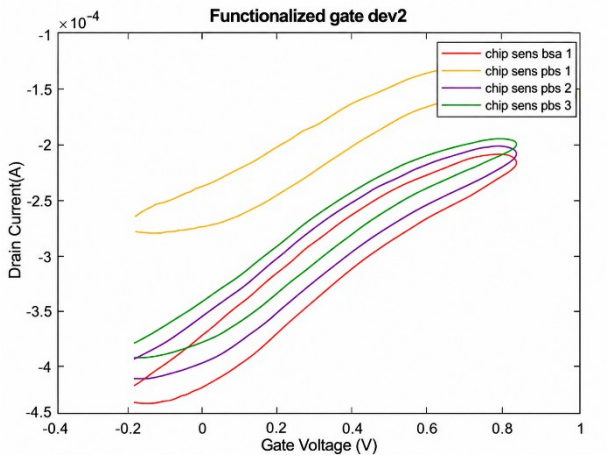


Figure 18: Transfer curves acquired on device dev2 using the anti-IL-6-functionalized gate during the third experimental campaign, under the different measurement conditions involving PBS and BSA-containing solutions.

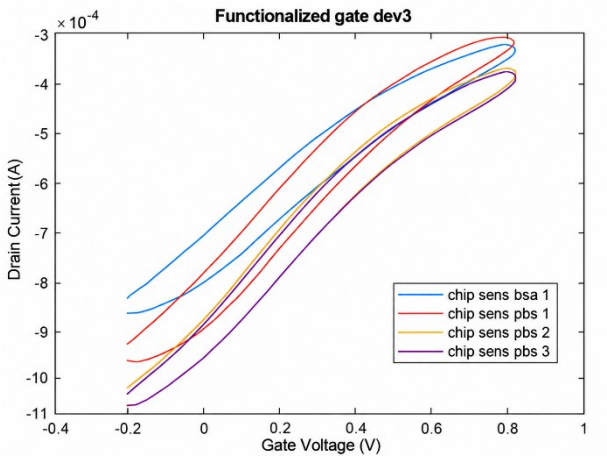


Figure 19: Transfer curves acquired on device dev3 using the anti-IL-6-functionalized gate during the third experimental campaign, under the different measurement conditions involving PBS and BSA-containing solutions.

For fourth campaign, the non-functionalized control electrode, although it had been prepared, was not subjected to measurement, so the comparison with the functionalized gate based on the specificity control adopted in the previous campaigns is not available for these data.

Transfer curves were acquired on the three available devices (dev1, dev2, dev3), exposing the functionalized gate to the two IL-6 solutions (10 nM and 2 nM) and to the 0.01× PBS reference solution. For devices dev1 and dev2, the curves show a clear separation: measurements obtained in the presence of IL-6 (at both concentrations) are placed at a lower current level than PBS-only repeats which are at a higher current level. This separation represents the first indication of a possible systematic difference associated with the composition of the solution in contact with the gate. For device dev3, on the other hand, only a single curve was acquired, of markedly lower amplitude than that recorded on the other two devices; the absence of further comparison measurements on this device does not allow any conclusion to be drawn regarding a possible specific response to the presence of IL-6.

The time evolution of the drain current for device dev1 was also recorded (dev1 vs Time), showing an overall increasing trend, characterized by a slow, continuous drift superimposed on a noise component, without the abrupt step change observed instead in the first campaign.

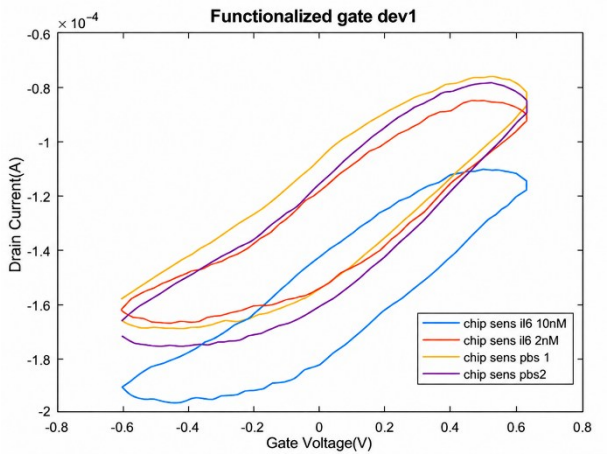


Figure 20: Transfer curves acquired on device dev1 using the anti-IL-6-functionalized gate during the fourth experimental campaign, in the presence of IL-6 at 10 nM and 2 nM and the PBS reference solution.

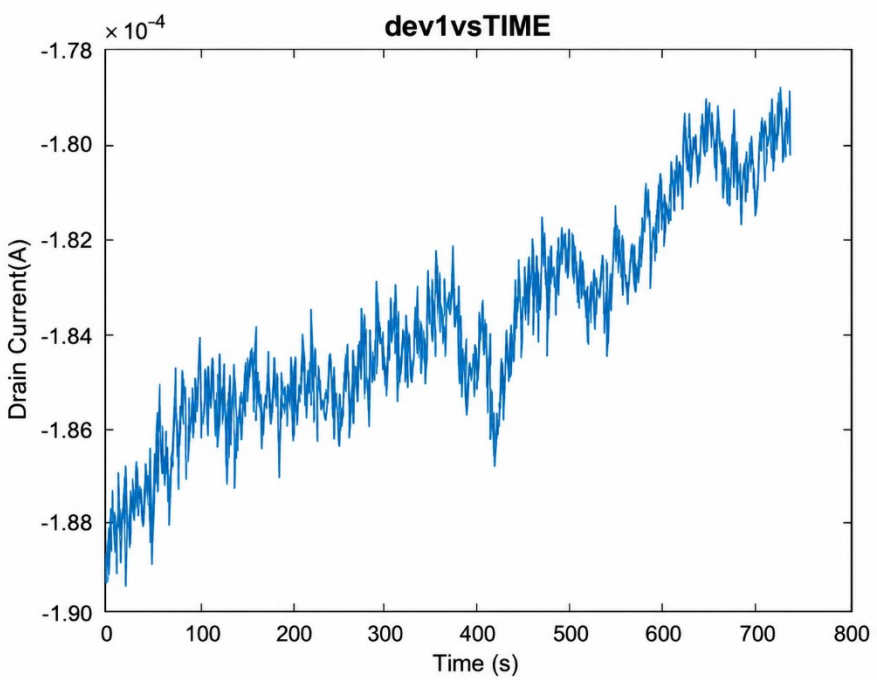


Figure 21: Time evolution of the drain current recorded for device dev1 during the fourth experimental campaign, showing an overall increasing trend characterized by a gradual drift and superimposed signal fluctuations.

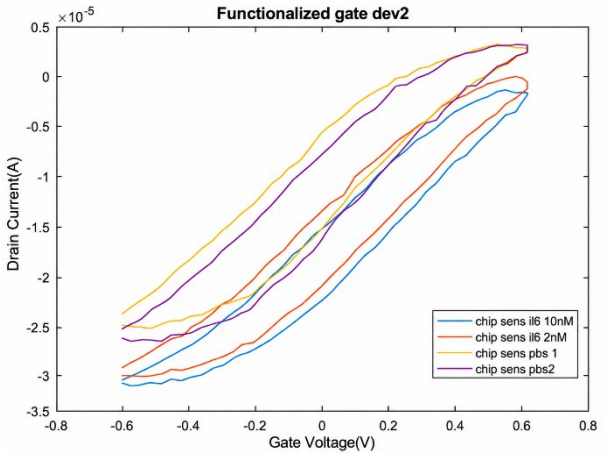


Figure 22: Transfer curves acqu during the fourth experimental campaign, in ıce solution.

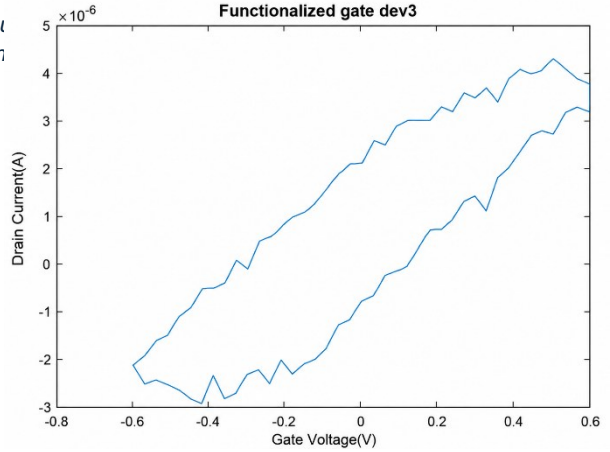


Figure 23: Transfer curve acquired on device dev3 using the anti-IL-6-functionalized gate during the fourth experimental campaign.

Overall, the analysis of the data collected across the four experimental campaigns shows that, in most cases, the functionalized OECT system did not provide an electrical response that was clearly and systematically distinguishable as a function of the presence of IL-6 in the solution in contact with the gate.
A partial exception is represented by the fourth campaign, in which the curves obtained in the presence of IL-6 show, for two of the three devices analysed, a more recognizable separation from the reference solution. This observation must nevertheless be considered with caution, since in this campaign it was not possible to perform the measurement on the control electrode, an element that in the previous campaigns had made it possible to verify the specificity of the response with respect to the mere electrical behaviour of the system.
These results, taken together, will be revisited and discussed in greater depth in the following chapter, in which an attempt will be made to correlate the evolution of the experimental conditions adopted with the trend of the observed response.

3.4 Potential and Limitations of OECT Technology

The studies discussed in the previous sections, together with the experimental results presented in this chapter, allow some broader considerations to be drawn regarding the potential and current limitations of OECT technology as a platform for cytokine biosensing.
From the perspective of its potential, the most distinctive feature of OECTs remains their intrinsic signal amplification capability, which arises from the volumetric coupling between ionic and electronic charges within the transistor channel [13,42]. This property allows a relatively small number of binding events occurring at the

gate electrode to produce a measurable variation in the drain current, without the need for external amplification circuitry. When combined with high-density and properly oriented bioreceptor layers, this mechanism has enabled extremely high sensitivities to be achieved. Preziosi et al. demonstrated the detection of TG2 protein down to attomolar concentrations, specifically 13 aM, using OECTs with gates functionalized with anti-TG2 antibodies [14], whereas Barra et al. reported the detection of the SARS-CoV-2 Spike protein in the femtomolar range through the analysis of transconductance variations [15]. Particularly high performance was also achieved by Guo et al. using nanobody-functionalized OECTs, which exhibited a detection range extending from the attomolar to the nanomolar regime and were capable of achieving single-protein-molecule detection [42]. These findings highlight the potential of OECTs for detecting analytes at extremely low concentrations, although such performance strongly depends on the device architecture, functionalization strategy and experimental conditions employed. In several cases, these sensitivity levels are comparable to or even higher than those achieved with conventional immunoassays such as ELISA, while requiring smaller sample volumes and shorter incubation times.

A second important advantage lies in the versatility of gate functionalization strategies developed for OECT biosensors. In addition to the well-established use of antibodies, alternative recognition elements such as aptamers [44], nanobodies [42] and zwitterionic antifouling polymers [43] have been successfully integrated at the gate–electrolyte interface, each offering different advantages in terms of cost, stability and ease of synthesis. In particular, the antibody-free approach based on phosphorylcholine-functionalized PEDOT proposed by Qian et al. demonstrates how gate chemistry can be designed not only to provide molecular recognition, but also to intrinsically resist the nonspecific adsorption of proteins, thereby addressing one of the most persistent sources of background signal in immunosensing [43]. This modularity implies that, in principle, the same basic transistor architecture could be adapted to different analytes simply by modifying the gate functionalization, without requiring a complete redesign of the device.

Despite these favorable characteristics, the literature reviewed also highlights a number of limitations, which emerged more clearly in the experimental work described in the previous sections of this chapter. A first limitation concerns the large number of interdependent parameters that must be optimized to achieve a reliable response, including the chemistry of the self-assembled monolayer (SAM), the density and orientation of the immobilized bioreceptor, the choice of blocking agent, the composition of the electrolyte and the specific gate-voltage window considered for data analysis [13–15]. Several of the studies discussed above explicitly reported that only a narrow voltage window, or a specific parameter related to transconductance, was sensitive to the antigen–antibody interaction, whereas the overall modulation of the current could also be influenced by nonspecific phenomena [14,15]. In the present work, the successive modifications introduced across the four measurement campaigns, including changes in buffer composition, the introduction of drying steps and the presence or absence of

blocking treatment with ethanolamine, reflect the same underlying difficulty. In the absence of an established reference protocol for the specific analyte and device architecture investigated, the functionalization procedure must be empirically refined through successive optimization steps.

A second limitation, closely related to the previous one, concerns reproducibility among different devices and across successive measurement sessions. Even in studies reporting excellent sensitivity in buffer solutions, device-to-device variability and stochastic effects at the lowest analyte concentrations are acknowledged as unresolved issues that complicate the translation of these sensors into quantitative diagnostic tools [42]. Detailed kinetic analyses performed on antibody-functionalized organic electrolyte-gated transistors have further shown that the relationship between the observed kinetic constant and analyte concentration does not always follow the linear behavior predicted by the simplest binding models. This behavior has been attributed to the coexistence, on the gate surface, of heterogeneous populations of binding sites with different affinities for the target. These findings suggest that part of the variability observed in OECT biosensors may arise not only from instrumental or fabrication-related factors, but also from the intrinsically heterogeneous nature of the biorecognition layer [45].

This issue also emerged clearly during the experimental campaigns conducted in the present work. In three of the four campaigns, the transfer curves recorded in the presence of IL-6 could not be clearly distinguished from those obtained using the reference solution alone, while a comparable degree of variability was also observed for the non-functionalized control electrode. Only in the fourth campaign was a slightly clearer separation observed; however, even in this case, the absence of a parallel control measurement limited the possibility of unequivocally attributing the observed variation to the presence of IL-6.

A further limitation concerns the transition from measurements performed in simple buffer solutions to more complex biological matrices, such as serum, plasma, or saliva. Although several of the OECT immunosensors discussed above maintained good sensitivity when tested in saliva or serum spiked with the target protein [15,42], the review by Burtscher et al. highlights how the nonspecific adsorption of the numerous proteins present in real biological fluids remains, together with the limited long-term stability of antibodies, one of the major obstacles to translating cytokine OECT biosensors into Point-of-Care applications [13]. Antifouling strategies, such as the zwitterionic gate coating discussed above, represent a promising approach to addressing this issue; however, they have so far been validated mainly for individual target proteins and under controlled laboratory conditions [43].

Overall, these considerations indicate that OECT technology combines a genuinely distinctive sensing mechanism, capable, in appropriately optimized configurations, of reaching the attomolar concentration range and, in specific nanobody-based systems, even single-protein-molecule detection [14,42], with a number of practical challenges that are still actively being addressed by the scientific community. The gap between the performance achieved under optimized experimental conditions

and the more variable behavior observed when moving toward complex biological matrices, non-specialized operators, or less extensively characterized device batches is consistent with the exploratory nature of the results obtained in the present work for IL-6 detection. This gap cannot be attributed exclusively to an intrinsic limitation of the technology, but should also be considered in light of the current stage of development of OECT-based immunosensors. Standardized functionalization protocols, systematic reproducibility studies involving multiple devices and validation using real clinical samples therefore remain essential steps before a direct comparison with established Point-of-Care platforms, such as the one discussed in Chapter 2, can become fully meaningful.

Chapter 4

General Discussion

4.1 Strengths and Weakturnnesses of Each Approach

The experimental results obtained in this work provide the basis for a broader consideration of the two approaches investigated for IL-6 detection. The Finecare™ device confirmed, in the experimental validation described in Chapter 2, the typical advantages of fluorescence-based Point-of-Care systems already on the market. The first advantage is speed: the Standard and Quick modes give a quantitative result in times suited to decentralized use, without requiring complex sample preparation. The short turnaround time makes this platform particularly suitable for environments in which timely diagnostic information is required, such as emergency departments, intensive care units, outpatient clinics, or healthcare facilities without immediate access to centralized laboratory services. These features represent some of the major advantages of Point-of-Care systems and explain their increasing adoption in routine clinical practice [46].
The second advantage is ease of use: the system can be used even by non-specialized staff. This aspect is also confirmed by another study that validated the same instrument for a different analyte, procalcitonin, using it directly at the patient's bedside in intensive care, with analysis times within 30 minutes [34].
The third advantage is good repeatability: consecutive Quick measurements showed low coefficients of variation, often below 3%, in line with what the manufacturer

states for the IL-6 kit (CV ≤15% both between repeated measurements and between different lots) [38]. The stated measurement range is also wide (3–4000 pg/mL, with a detection limit ≤3 pg/mL) and no hook effect is observed within the linear range, which reduces the need to dilute the sample [38]. Alongside these strengths, however, the experimental validation revealed an important limitation: the systematic underestimation of known IL-6 concentrations, with an extent that varies greatly depending on the matrix. Mean recovery was about 8% in saline solution and about 63% in plasma. This difference shows that the system is sensitive to matrix composition, an aspect to consider especially near the lower measurement limit (about 3 pg/mL), where small absolute variations carry a large relative weight. It is worth noting that such a marked bias is not necessarily a general limitation of the instrument: in the procalcitonin study, the comparison between whole blood on Finecare™ and plasma on the reference method (Cobas e411) gave a small mean bias (0.22 ng/mL) and excellent agreement (ICC = 0.979) [34]. This suggests that the deviation observed for IL-6 depends more on the specific kit and analyte — including possible interference from plasma components — than on a general limitation of the fluorescence-based technology itself. The instructions for use of the IL-6 kit, in fact, list several substances that can interfere with the result (CRP, serum amyloid A, procalcitonin, troponin I, other interleukins, hemoglobin, total protein, bilirubin, triglycerides, cholesterol, rheumatoid factor, heterophilic antibodies and certain drugs) above certain concentration thresholds, with a stated deviation of up to ±15% [38]. These thresholds alone do not explain the underestimation observed, but they do confirm that the assay is sensitive to the composition of the biological matrix. One final issue, observed but not systematically confirmed, was a progressive increase in values across some series of consecutive Quick measurements on the same sample. This suggests further investigation into signal stability over time, possibly linked to the device's sensitivity to factors such as vibration, magnetic fields, or variations in temperature and humidity, which are mentioned among the manufacturer's precautions for use [38].

The OECT system remains technologically interesting for the principles described: volumetric ion-electron signal amplification, operation at low voltages and the ability to functionalize the gate without affecting the organic channel that carries the charge. Unlike an optical readout test such as Finecare™, where the signal is amplified by the reading instrument, in the OECT amplification is an intrinsic property of the device itself. This opens up, in theory, the possibility of very high sensitivity — down to the attomolar level — without the need for additional electronics. These advantages are documented in the literature for other OECT protein-detection applications as well [13,42,44] and make the technology scalable toward miniaturized, low-power devices, a useful feature for future Point-of-Care use. Another strength is the variety of strategies available for functionalizing the gate — antibodies, nanobodies, aptamers, antifouling polymers — which would, in theory, allow the same basic architecture to be adapted to different analytes simply by changing the surface chemistry, without redesigning the whole device. Neither of the other two platforms considered here (optical or electrochemical) offers this

same flexibility. These theoretical advantages, however, come up against significant practical limitations in the present work. The most evident one, present in most of the four experimental campaigns, is the absence of an electrical response clearly and repeatedly linked to the presence of IL-6. Added to this is strong variability between different devices, which required the use of multiple units (dev1, dev2, dev3) over the course of the campaigns. This variability is not unique to the present work: it is a known problem in the literature too. Kinetic studies on antibody-functionalized OECTs have shown that the relationship between the observed kinetic constant and analyte concentration is not always linear, a behavior attributed to the presence on the gate of binding sites with different affinities from one another [45]. This factor, together with the variability observed at low concentrations in other OECT immunosensors as well [42], suggests that part of the irreproducibility observed depends on the uneven nature of the bio-recognition layer and not only on instrumental or fabrication factors. Another limitation is the lack of an established reference protocol for this analyte and this device architecture and is also connected to the stability and biofouling issues reported in the literature for this class of devices when applied to complex biological matrices [13,43].

4.2 Improvement Proposals and Future Applications

The results obtained in the present work have identified several aspects that deserve further investigation. Although the Finecare™ FIA Meter Plus and the OECT platform currently represent technologies at different stages of development, both offer opportunities for improvement that could enhance their reliability and broaden their potential fields of application.

With regard to the Finecare™ system, future studies should include systematic experimental validation against an established reference methods in order to better understand the origin of the underestimation observed in the present study and to assess the possible contribution of factors such as sample matrix, sample preparation and other experimental variables. An additional step towards a more comprehensive evaluation would be the assessment of the device under real clinical conditions, using samples collected from patients presenting different pathological conditions.

Even broader opportunities for improvement emerge in the case of the OECT platform. The experience gained throughout the four experimental campaigns demonstrated that obtaining a stable and reproducible electrical response depends on the simultaneous optimization of multiple parameters. Among these, the functionalization of the gate surface appears to be one of the most critical aspects. Future investigations could therefore focus on optimizing incubation times, reagent concentrations and biofunctionalization strategies in order to improve the reproducibility of the modified surface and to promote a more efficient interaction between the immobilized bioreceptor and the target analyte. As highlighted by Burtscher et al. [13], the performance of OECT-based biosensors is strongly

influenced by the properties of the gate/electrolyte interface, making careful control of the functionalization process essential from the earliest stages of device preparation.
Another important aspect concerns the characterization of the surface throughout the different functionalization steps. The integration of complementary surface characterization techniques would make it possible to verify the successful formation of the self-assembled monolayer, the effective immobilization of the antibody and the homogeneity of the modified surface before electrical measurements are performed. Such characterization would facilitate the identification of possible shortcomings in the functionalization process and improve its reproducibility, allowing chemical issues to be distinguished from limitations associated with the electrical response of the device.
Further optimization should also address the measurement conditions. Throughout the present work, electrical signal instability emerged as one of the main factors limiting the interpretation of the experimental results. Future studies should therefore investigate the influence of electrolyte composition, environmental conditions, device geometry and measurement parameters on the electrical behaviour of the OECT, with the aim of reducing measurement variability and improving the signal-to-noise ratio. As reported in the literature, even minor variations in the operating conditions may significantly affect transistor performance and, consequently, the sensitivity of the biosensor [13].
Another promising research direction involves the exploration of alternative molecular recognition strategies. Although the present study relied on the immobilization of anti-IL-6 antibodies, recent investigations have demonstrated that aptamers and nanobodies may represent effective alternatives, offering advantages in terms of stability, reproducibility and, in some cases, analytical sensitivity [42,44]. In particular, Diacci et al. [44] developed an OECT aptasensor for IL-6 detection, demonstrating the feasibility of an antibody-free recognition strategy, whereas Guo et al. [42] showed that nanobody-functionalized OECTs can achieve extremely high sensitivity, enabling the detection of very low biomarker concentrations. Although these approaches require further comparative investigation, they represent promising alternatives that could also be explored in future developments of the sensing platform investigated in the present work.
In parallel, future studies should extend the experimental validation to real biological matrices. Following the preliminary optimization performed under controlled laboratory conditions, the sensing platform should be evaluated using plasma, serum, or other biological fluids containing known concentrations of IL-6 in order to investigate the effects of sample matrix, biological interference and intrinsic sample variability on sensor performance. This step represents one of the key requirements for translating a biosensing technology from laboratory research to clinical application [13].
From a broader perspective, the future development of OECT technology could also benefit from integration with microfluidic systems, miniaturized electronics and portable data acquisition and processing platforms. The combination of these

technologies may facilitate the development of increasingly compact, automated and user-friendly Point-of-Care devices, thereby expanding their potential applications in both clinical diagnostics and decentralized patient monitoring.
Indeed, the intrinsic characteristics of OECTs, including low-voltage operation, inherent signal amplification and compatibility with flexible substrates, make this technology particularly attractive for the development of next-generation miniaturized biosensors [13].

Chapter 5

Conclusions

5.1 Summary of Key Results

This thesis set out two main objectives: to select and experimentally validate a commercial Point-of-Care device for biomarker determination and to carry out a preliminary study on an experimental OECT device for the detection of the same class of analytes, with particular attention to interleukin-6 (IL-6).
Regarding the commercial device, after a comparative market analysis the Finecare™ FIA Meter Plus was selected, together with the dedicated IL-6 kit. The experimental validation, carried out first through exploratory tests and then through a more systematic protocol on samples with known concentrations prepared in saline solution and in plasma, showed a device capable of providing a response consistently related to the IL-6 concentration in the sample, with good repeatability between consecutive measurements (coefficients of variation almost always below 3%). At the same time, a systematic underestimation of known concentrations emerged, considerably more pronounced in saline (mean recovery 8.42%) than in plasma (mean recovery 63.17%). It is confirmed that the sample matrix has a significant inconsistency in measurement accuracy. From a practical point of view, the device proved to be easy to use, short analysis times (15 minutes) and relatively low cost compared to centralized laboratory instrumentation (about €3,750 for the analyzer, about €13 for a single IL-6 test).

For the OECT system, the work focused on gate functionalization with anti-IL-6 antibodies and on four successive measurement campaigns, carried out on multiple devices and with increasing IL-6 concentrations, from the picomolar to the nanomolar level. In most cases, the curves obtained in the presence of IL-6 were not clearly distinguishable from those obtained with the reference solution alone, nor from the behaviour of the non-functionalized control electrode; in addition, marked variability between different devices was observed.
The OECT, on the other hand, represents a platform still at the stage of methodological development, for which the functionalization protocol, device-to-device reproducibility and validation on real biological matrices still need to be consolidated before a direct comparison with established Point-of-Care platforms can become truly meaningful.

5.2 Thesis Contributions and Future Developments

The main contribution of this work was to examine, for the same analyte and with the same application goal, two technologies at very different stages of maturity: a commercial device already ready for use and an experimental platform still under development.
With regard to the Finecare™, the contribution of the thesis is primarily practical: the quantitative characterization of recovery and bias across two different matrices provides concrete data, not readily available in the manufacturer's documentation, on how much trust can be placed in the absolute values returned by the instrument for IL-6 and on how much the choice of matrix matters when interpreting a result.
For the OECT, the contribution is of a different nature: rather than a performance figure, the thesis provides a map of the real difficulties encountered in bringing a device of this kind from theory to practical measurement, information that is useful to anyone continuing this work, even in the face of an inconclusive experimental outcome.
Regarding future developments, the specific technical directions for improving the two platforms have already been discussed and are not repeated here. In more general terms, the way forward for the Finecare™ is to extend the validation to a real clinical case series, with a direct check against a reference laboratory method; for the OECT, the next step is to establish a stable and reproducible functionalization protocol, as a necessary basis before any attempt at quantitative validation.
Looking beyond the two specific devices, this work fits within a broader path toward decentralized diagnostics, in which already mature tools such as the Finecare™ and still emerging technologies such as the OECT coexist and complement one another: the former meet an immediate clinical need, while the latter represent the investment on which the next generation of biosensors could be built.

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Appendices

Appendix 1

Solution	Preparation	Final Volume	Concentration
Stock 1	5 µg IL-6 + 10 µL Milli-Q water	10 µL	0.5 mg/mL
Stock 2	1 µL of Stock 1 + 49 µL PBS containing 0.1% BSA	50 µL	10 µg/mL
Stock 3	Dilution of Stock 2 in PBS	2 mL	0.25 µg/mL
Measurement solution	Serial dilutions of Stock 3	Variable	2 pM (≈ 47.4 pg/mL)

Appendix 2

Solution	Composition	Final Concentration
Solution 1	PBS 1×	—
Solution 2	PBS + BSA	0.1% BSA
Measurement solution	PBS + BSA + IL-6	10.5 nM IL-6; 375 nM BSA (≈ 0.0025% w/v)

Appendix 3

Solution	Composition	Final Concentration
Solution 1	PBS 0.01×	—
Solution 2	PBS 0.01× + BSA	0.1% BSA
Measurement solution	PBS + BSA + IL-6	10.5 nM IL-6; 375 nM BSA (≈ 0.0025% w/v)

Appendix 4

Solution	Preparation	Final Volume	Concentration
Stock 1	100 µg lyophilized IL-6 + 500 µL Milli-Q H_2O	500 µL	0.2 mg/mL
Stock 2	50 µL Stock 1 + 150 µL PBS	200 µL	0.05 µg/µL (50 µg/mL)
Measurement solution A	10 µL Stock 2 + PBS up to volume	2 mL	≈ 10.5 nM
Measurement solution B	2 µL Stock 2 + PBS up to volume	2 mL	≈ 2.1 nM