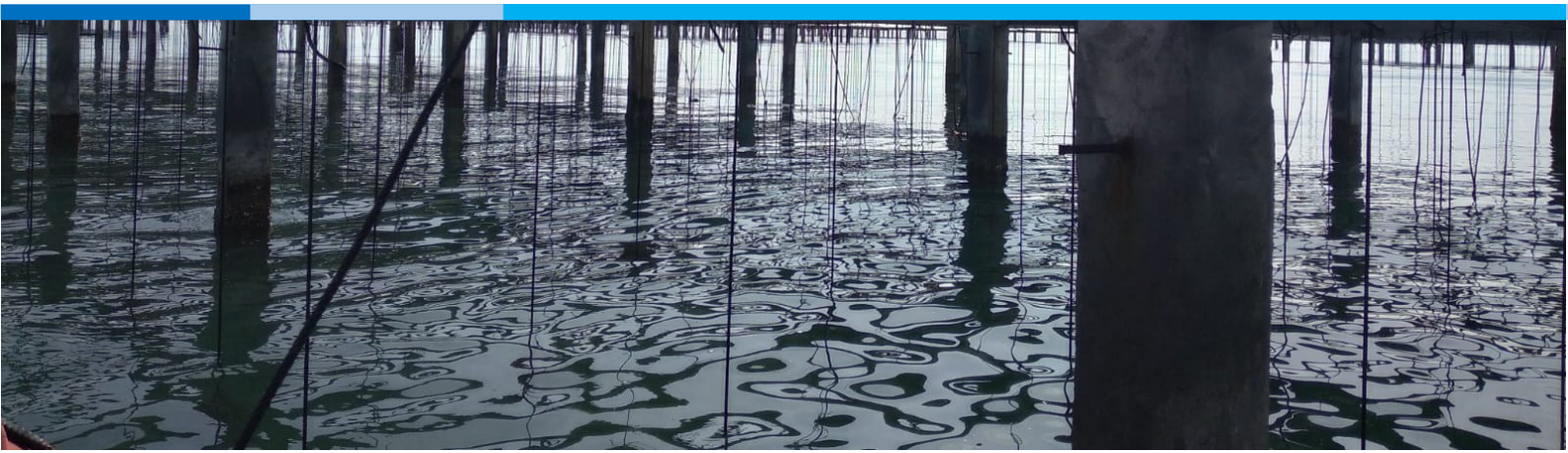




# XXVIII TRANSFRONTIER MEETING ON SENSORS AND BIOSENSORS (TMSB2024)

## BOOK OF ABSTRACTS

IRTA La Ràpita | 26 - 27 September 2024





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de **BIOLOGIA**



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**HOSTING INSTITUTION:**

**Institute of Agrifood Research and Technology (IRTA)**

*Ctra. Poble Nou, km 5.5*

*43540 La Ràpita*

**ORGANISING COMMITTEE:**

Mònica Campàs

Jaume Reverté

Romina Ventura

Mounira Alkassar

Sandra Leonardo

## BENVINGUTS AL TMSB 2024!

Dear colleagues,

We are thrilled and honoured to welcome you to the 28<sup>th</sup> edition of the Transfrontier Meeting on Sensors and Biosensors (TMSB 2024). Established in 1996 by Prof. Salvador Alegret (Universitat Autònoma de Barcelona), Prof. Jean-Louis Marty (Université de Perpignan-Via Domitia), and Prof. Maurice Comtat (Université Paul Sabatier), TMSB was conceived with the goal of fostering collaboration among researchers from the Euroregion (Catalonia, Occitanie) working in the field of sensors and biosensors. Its primary mission is to create new partnerships and provide a platform for young scientists to share their work.

Over the years, TMSB has been hosted alternately by research groups from both sides of the border. This year, we are excited to hold the meeting at the Institute of Agrifood Research and Technology (IRTA) in La Ràpita, organised by the Biosensors group led by Dr. Mònica Campàs.

- |  |   |
|--|---|
| 1 – Barcelona, 15-16 July 1996                   | 15 – Sant Carles de la Ràpita, 16-17 September 2010       |
| 2 – Céret, 18-19 September 1997                  | 16 – Toulouse, 29-30 September 2011                       |
| 3 – Peníscola, 17-18 September 1998              | 17 – Tarragona, 20-21 September 2012                      |
| 4 – Montpellier, 16-17 September 1999            | 18 – Alès, 19-20 September 2013                           |
| 5 – Vic, 21-22 September 2000                    | 19 – Barcelona, 25-26 September 2014                      |
| 6 – Toulouse, 20-21 September 2001               | 20 – Perpignan, 1-2 October 2015                          |
| 7 – Barcelona, 19-20 September 2002              | 21 – Barcelona, 29-30 September 2016                      |
| 8 – Céret, 18-19 September 2003                  | 22 – Montpellier, 21-22 September 2017                    |
| 9 – Tarragona, 16-17 September 2004              | 23 – Barcelona, 19-20 September 2018                      |
| 10 – Albi, 15-16 September 2005                  | 24 – Perpignan, 26-27 September 2019                      |
| 11 – Girona, 14-15 September 2006                | 25 – Sant Carles de la Ràpita (online), 30 September 2021 |
| 12 – Céret, 27-28 September 2007                 | 26 – Barcelona, 29-30 September 2022                      |
| 13 – Andorra-la-Vella, 18-19 September 2008      | 27 – Banyuls de la Marenda, 28-29 September 2023          |
| 14 – Banyuls de la Marenda, 24-25 September 2009 | <b>28 – La Ràpita, 26-27 September 2024</b>               |

True to its spirit, TMSB offers a relaxed and friendly atmosphere, prioritising the participation of predoctoral researchers and young scientists, who will present their findings through oral and poster sessions. We are confident that this meeting will spark fruitful scientific exchanges and deepen the connections between the research institutions involved.

We look forward to an inspiring and productive event!



**Mònica Campàs**  
*on behalf of the Organising Committee*



# SCIENTIFIC AND SOCIAL PROGRAMME

| XXVIII Transfrontier Meeting on Sensors and Biosensors (TMSB 2024) |   |   |
|--|---|---|
| Scientific and Social Programme                                    |   |   |
| Thursday 26 <sup>th</sup> September                                |   |   |
| 14:00 – 14:50  | Registration and Poster Installation  |   |
| 14:50 – 15:00  | TMSB2024: Opening Session   |   |
| 15:00 – 16:30  | <b>Oral Presentations: First Session</b><br>Biorecognition Elements for Biosensing<br>Chairman: <b>Thierry Noguer (UPVD)</b>  |   |
| 15:00 – 15:30  | <b>Anna Toldrà (KI)</b><br>Decentralizing nucleic acid tests: Towards integrated devices using paper, textile and electronics   | KN 01   |
| 15:30 – 15:45  | <b>Shaira Jane Acosta (URV)</b><br><i>In vitro</i> selection of DNA aptamers for highly specific recognition of <i>Mycobacterium tuberculosis</i> ESAT-6/CFP-10 heterodimer antigen     | OC 01   |
| 15:45 – 16:00  | <b>Elena Rodríguez-Franch (UAB)</b><br>Improving cocaine voltammetric detection with molecularly imprinted polymer modified sensor  | OC 02   |
| 16:00 – 16:15  | <b>Jaume Reverté (IRTA)</b><br>The importance of antibody cross-reactivity in immunoanalytical tools for toxins   | OC 03   |
| 16:15 – 16:30  | <b>Mounira Alkassar (IRTA)</b><br>Neuroblastoma cell-based tools and HPLC-FLD for the detection of paralytic shellfish toxins in marine pufferfish from the Spanish Mediterranean coast | OC 04   |
| 16:30 – 17:15  | <b>Coffee break</b><br><b>Poster Session (PC 01 – PC 21)</b>  |   |
| 17:15 – 18:15  | <b>Oral Presentations: Second Session</b><br>New Materials and Sensor Platforms<br>Chairwoman: <b>Mar Puyol (UAB)</b>   |   |
| 17:15 – 17:30  | <b>Franc Paré (UAB)</b><br>3D-printing technology for unconventionally shaped electrode's fabrication   | OC 05   |
| 17:30 – 17:45  | <b>Deepanshu Verma (ICIQ)</b><br>Porous alumina-based biosensing platforms for early-stage infection diagnosis  | OC 06   |
| 17:45 – 18:00  | <b>Roger Serentill-Grau (UAB)</b><br>Development of electrochemical sensors with 3D printing technology for the analysis of illicit substances  | OC 07   |
| 18:00 – 18:15  | <b>Marwa Ben Amar (IMB-CNM)</b><br>Smart analytical microtechnology for aquaculture process monitoring  | OC 08   |
| 20:00  | <b>Gala Dinner</b><br><b>Restaurant Juanito Platja</b><br>Passeig Marítim, 50, 43540 La Ràpita<br>(Scan the QR code for on-screen map instructions)                                     |  |

| XXVIII Transfrontier Meeting on Sensors and Biosensors (TMSB 2024) |   |   |
|--|---|---|
| Scientific and Social Programme                                    |   |   |
| Friday 27 <sup>th</sup> September                                  |   |   |
| 09:00 – 10:00  | <b>Oral Presentations: Third Session</b><br>Portable Devices for Point-of-Care Applications<br>Chairman: <b>Julio Bastos-Arrieta (UB)</b>   |   |
| 09:00 – 09:30  | <b>Manuel Gutiérrez-Capitán (IMB-CNM)</b><br>Point-of-care (PoC) devices based on multiplexed electrochemical biosensors for early disease diagnosis                                  | <b>KN 02</b>  |
| 09:30 – 09:45  | <b>Anaïxis del Valle (UAB)</b><br>Portable PCR and electrochemical genosensing for the rapid screening test development to detect neonatal <i>Streptococcus agalactiae</i> infections | <b>OC 09</b>  |
| 09:45 – 10:00  | <b>Juan Carlos Porras (UAB)</b><br>Portable medical device based on magneto immunosensor for the diagnosis of celiac disorder   | <b>OC 10</b>  |
| 10:00 – 10:45  | <b>Coffee break</b><br><b>Poster Session (PC 01 – PC 21)</b>  |   |
| 10:45 – 11:30  | <b>Oral Presentations: Fourth Session</b><br>Sensing Tools for Emerging Contaminants<br>Chairwoman: <b>Beatriz Prieto-Simón (ICIQ)</b>  |   |
| 10:45 – 11:00  | <b>Youssef Ali (CNIEL, ANSES)</b><br>Colorimetric and electrochemical enzymatic biosensor for the detection of disinfectants in dairy products  | <b>OC 11</b>  |
| 11:00 – 11:15  | <b>Jing Huang (UB)</b><br>Electrochemical determination of emerging contaminants using tailored layer-by-layer modification of screen-printed carbon electrodes                       | <b>OC 12</b>  |
| 11:15 – 11:30  | <b>Ulises Guillermo Díaz Avello (URV, IRTA)</b><br>Accurate and fast tetrodotoxin detection by a paper-based test based on an antibody-aptamer sandwich assay                         | <b>OC 13</b>  |
| 11:30 – 11:45  | <b>Final Remarks and TMSB2024 Awards</b>  |   |
| 12:00 – 16:00<br>(tentative)                                       | <b>Boat Excursion and Closure Lunch</b><br><b>Musclarium</b><br><i>Badia dels Alfacs, Port Esportiu, 43540 La Ràpita</i><br><i>(Scan the QR code for on-screen map instructions)</i>  |   |
|  |   |  |



# LIST OF POSTERS



| XXVIII Transfrontier Meeting on Sensors and Biosensors (TMSB 2024) |  |
|--|--|
| List of Posters  |  |
| PC 01  | <b>Jennifer Marfà (UAB)</b><br>Integration of molecularly imprinted polymers into paper-based nucleic acid lateral flow platforms for enhanced multiplexed point-of-need diagnostics |
| PC 02  | <b>Alexandros Lazanas (ICIQ)</b><br>Coupled size-exclusion and electrocatalytic activity of CuO QDs functionalized porous silicon electrodes directly used in human saliva           |
| PC 03  | <b>Rafael C. Hensel (UAB, USP)</b><br>Laser-induced graphene electrodes for wearable sweat sensors   |
| PC 04  | <b>Xavier Cetó (UAB)</b><br>Inkjet-printed sensor array for the simultaneous detection of drugs of abuse adulterants   |
| PC 05  | <b>Jaume Reverté (IRTA)</b><br>The BLUESHELLFISH project: Combination of toxicological and structural-recognition data for ciguatera risk assessment                                 |
| PC 06  | <b>Maria Trachioti (ICIQ)</b><br>Development of 3D-printed micropillar-based electrochemical sensors   |
| PC 07  | <b>Eva Arasa-Puig (UAB)</b><br>Microanalyzer for pH determination in wine  |
| PC 08  | <b>Marta Meneghello (UPVD)</b><br>Development of a biosensor to detect cellular heterogeneity during fermentation  |
| PC 09  | <b>Laia Garrido Carretero (UAB)</b><br>Towards the industrialization of disposable biomedical devices for at-home use  |
| PC 10  | <b>Laia Garrido Carretero (UAB)</b><br>Disposable device for at-home alanine blood monitorization for controlling mitochondrial diseases   |
| PC 11  | <b>Fearghal O'Connor (URV, ICIQ)</b><br>Porous silicon-based sensor to detect and monitor biofilm growth   |
| PC 12  | <b>Martina Tolós (IRTA)</b><br>Visual test for the detection of $\gamma$ -glutamyl transpeptidase in calf serum  |
| PC 13  | <b>Antonio Calvo-López (IRSJD)</b><br>Characterization of microfluidic platforms for their application in assisted reproduction processes  |
| PC 14  | <b>Antonio Calvo-López (IRSJD)</b><br>Cadmium monitoring using automated microanalyzers in the hydrometallurgical production of zinc   |
| PC 15  | <b>Antonio Calvo-López (IRSJD)</b><br>Cobalt monitoring in the hydrometallurgical production of zinc using an automated spectrophotometric microanalyzer                             |
| PC 16  | <b>Olga Melisidou (UAB)</b><br>Evaluating the capability of pentamethincyanines as bactericides  |

| XXVIII Transfrontier Meeting on Sensors and Biosensors (TMSB 2024) |  |
|--|--|
| List of Posters  |  |
| PC 17  | <b>Melania Mesas (UAB)</b><br>Traffic light-based point-of-care test for the rapid stratification of fever syndromes   |
| PC 18  | <b>Julio Bastos-Arrieta (UB)</b><br>The evolving landscape of electroanalysis at the University of Barcelona   |
| PC 19  | <b>Ambbar Aballay-González (UdeC)</b><br>Detection of saxitoxin using an immunodetection tool based on magnetic beads  |
| PC 20  | <b>Yudong Bian (UAB)</b><br>Biochar based sustainable electrochemical screen-printed carbon sensors for environmental analysis: a perspective approach         |
| PC 21  | <b>Rosanna Rosi (UAB)</b><br><i>In-vitro</i> diagnostic test based on exosomes for early diagnosis of Alzheimer's disease and risk stratification of patients  |
| PC 22  | <b>Marianna Rossetti (ICN2)</b><br>Graphene-based nanomaterials integration with bioreceptors for enhanced point-of-care diagnostics and environmental sensors |
| PC 23  | <b>Nerea de Mariscal-Molina (ICN2)</b><br>Point-of-care haemoglobin detection for anaemia diagnosis  |
| PC 24  | <b>Davi de Farias (ICN2, USP)</b><br>Aptamer-based Inkjet-printed nanostructured biosensors for real-time environmental monitoring of antibiotics              |



**KEYNOTE  
COMMUNICATIONS**

## Decentralizing Nucleic Acid Tests: Towards Integrated Devices Using Paper, Textile and Electronics

A. Toldrà<sup>1,\*</sup>, S. Khaliliazar<sup>2</sup>, G. Chondrogiannis<sup>2</sup>, M. Hanze<sup>2</sup>, D. Barrett<sup>1</sup>, M.M. Hamed<sup>2</sup>, V. Pelechano<sup>1</sup>

<sup>1</sup> Karolinska Institutet, Department of Microbiology, Tumor and Cell Biology, Solna, Sweden.

<sup>2</sup> KTH Royal Institute of Technology, Department of Fiber and Polymer Technology, Stockholm, Sweden.

\*Correspondence: [anna.tf@scilifelab.se](mailto:anna.tf@scilifelab.se)

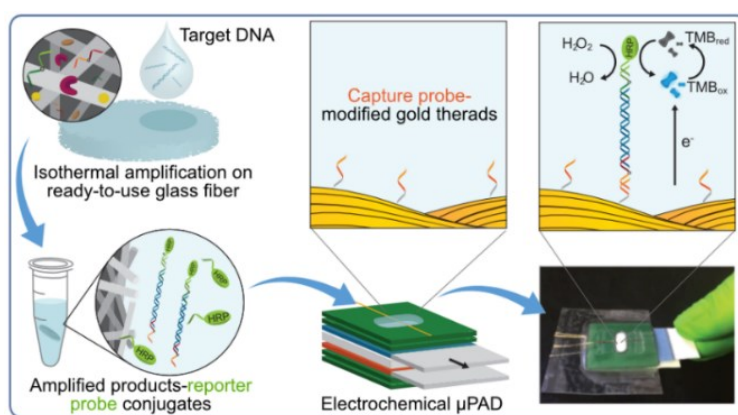
Nucleic acid amplification tests (NAATs) are the gold standard for diagnostics and various other applications, such as food safety and environmental monitoring, due to their high specificity and sensitivity. However, NAATs rely on benchtop instruments (e.g., qPCR) available only in centralized labs. Current efforts focus on decentralizing these tools to bring them closer to the point of need.

NAATs consist of three steps: sample preparation, DNA amplification, and DNA detection. While significant progress has been made in miniaturizing DNA amplification (e.g., isothermal amplification techniques) and detection methods (e.g., electrochemical readouts), sample preparation (e.g., cell lysis, DNA extraction) remains a bottleneck, hindering complete decentralization of these systems.

Additionally, to achieve fully integrated, sample-to-answer NAATs, all steps must be incorporated into microfluidic platforms that minimize user intervention. Paper and textiles are an affordable alternative to traditional microfluidics, enabling advanced functionalities such as capillary-driven fluid transport, filtration, and reagent storage. Specifically for electrochemical sensors, further integration into electronics is crucial. In this context, printed circuit boards (PCBs) offer scalable, cost-effective solutions compatible with electronic systems.

This presentation will highlight several approaches developed in the field, including: 1) vertical flow paper devices with enhanced sensitivity compared to conventional lateral flow assays <sup>[a]</sup> (**Figure 1**); 2) gold thread electrodes for electrochemical paper devices <sup>[b]</sup> and continuous analysis <sup>[c]</sup>; and 3) the integration of PCB-based sensors into open-source, hand-held potentiostats <sup>[d]</sup>. I will also present methods to improve sample preparation using enzyme immobilization on paper for cell lysis <sup>[e]</sup> and nuclease digestion <sup>[f]</sup>. I will conclude with an overview of my ongoing research, focusing on label-free impedimetric analysis <sup>[g]</sup> and multiplex detection strategies using molecular inversion probes.

**Keywords:** sample preparation, isothermal DNA amplification, paper-based devices, microfluidics.



**Figure 1.** Microfluidic paper-based electrochemical NAAT that exploits: 1) fibre substrates for storing isothermal amplification reagents and conducting the reaction; 2) movable stacks of filter paper for fluid transport and time-sequenced reactions; and 3) gold-coated threads that allow for SAMs formation, DNA hybridization, and amperometric detection.

**References:** <sup>a</sup> Toldrà A, et al. (2023) *Biotechnology Journal*; <sup>b</sup> Khaliliazar S<sup>†</sup>, Toldrà A<sup>‡</sup>, et al. (2022) *Analytical Chemistry*; <sup>c</sup> Hanze M, et al. (2023) *Biosensors*; <sup>d</sup> Toldrà A, et al. (2022) *Analyst*; <sup>e</sup> Chondrogiannis G, et al. (2021) *Scientific Reports*; <sup>f</sup> Chondrogiannis G, et al. (2024) *Advanced Material Interfaces*; <sup>g</sup> Tayyab M<sup>†</sup>, Barrett D<sup>‡</sup>, et al. (2023) *Science Advances*.

## Point-of-Care (PoC) Devices Based on Multiplexed Electrochemical Biosensors for Early Disease Diagnosis

M. Gutiérrez-Capitán, Á. Calleja, A. Baldi, C. Fernández-Sánchez\*

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Health initiatives worldwide demand affordable point-of-care devices to aid in the reduction of morbidity and mortality rates of high-incidence infectious diseases. However, the production of robust and reliable easy-to-use diagnostic platforms showing the ability to quantitatively measure several biomarkers in physiological fluids and that could in turn be decentralized to reach any relevant environment, remains a challenge. In fact, the recent Covid-19 pandemic evidenced the shortcomings of our healthcare systems in the massive deployment of low-cost point-of-care testing devices. There was a clear need for rapidly detecting virus infection, not only by identifying the virus infection agent but also by providing viral load numbers in order to give a more accurate information about the infection severity. Since 2017, the Chemical Transducers Group has been working on the development of this type of platforms based on the particular combination of paper-microfluidic technology, electrochemical transduction, and magnetic nanoparticle-based assay approaches to produce a unique, compact, and easily deployable multiplex device to measure clinical biomarkers. Unlike the common lateral-flow tests (LFT), this platform consists of an array of highly compact reusable electrochemical cells integrated in a polymeric cartridge, in combination with a disposable paper fluidic component. Electrochemical transducers and required electronics show well-known advantages for producing quantitative analytical platforms in terms of inherent small size, low cost, low power consumption, portability and high selectivity and sensitivity <sup>[a]</sup>. Fluidic devices made of cellulose materials have extensively been developed thanks to its great availability, low cost, safety in handling, inherent capillary flow, porosity and biocompatibility <sup>[b]</sup>. The implementation of magnetic nanoparticles (MNPs) in the operation of the device significantly improves the signal-to-noise ratio by being able to carry out more effective preconcentration and sample treatment steps.

The device was used to simultaneously measure interleukin-8, tumor necrosis factor- $\alpha$  and myeloperoxidase biomarkers in real sputum with the aim of facilitating the timely detection of acute exacerbations of chronic obstructive pulmonary disease (COPD). The analysis of samples of healthy individuals and acutely exacerbated patients produces statistically significant biomarker concentration differences between the two studied groups <sup>[c]</sup>. The device was also adapted to detect the presence of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) viral RNA within 40 minutes without the need for sample purification and gene amplification procedures. Nasopharyngeal swab samples collected from both PCR-positive and negative patients were used in a retrospective study to evaluate the device performance, and a sensitivity of 100% and a specificity of 93% were estimated by ROC analysis <sup>[d]</sup>.

**Keywords:** point-of-care rapid test, electrochemical biosensing, paper microfluidics, magnetic nanoparticle-based assay, multiplexed detection.

### References:

- <sup>a</sup> M. Gutiérrez-Capitán, et al. *Biosens. Bioelectron.* **2022**, *201*, 113952.
- <sup>b</sup> M. Gutiérrez-Capitán, et al. *Sensors* **2020**, *20*, 967.
- <sup>c</sup> M. Gutiérrez-Capitán, et al. *ACS Sensors* **2023**, *8*, 3032.
- <sup>d</sup> A. Aviñó, et al. *Int. J. Mol. Sci.* **2022**, *23*, 15258.



**ORAL  
COMMUNICATIONS**

## ***In vitro* selection of DNA aptamers for highly specific recognition of *Mycobacterium tuberculosis* ESAT-6/CFP-10 heterodimer antigen**

S. Jane Acosta<sup>1,\*</sup>, V. Skouridou<sup>1</sup>, C. O'Sullivan<sup>1,2</sup>

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<sup>2</sup>Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain.

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Despite the progress in rapid diagnostic techniques for tuberculosis (TB), non-sputum-based rapid tests remain a pressing need for TB detection. Antigen-based detection for TB in non-sputum samples presents prospects, but available and efficient approach in selecting bioreceptors limits the development of rapid tests due to batch variations, low stability and specificity. Antigenic proteins ESAT-6 and CFP-10, which form a tight 1:1 complex for increased stability and virulence, are considered good biomarkers for non-sputum-based detection, given that they are actively secreted by *M. tuberculosis* as a heterodimer and are strong predictors of TB infections. Herein, we describe the selection of DNA aptamers, which are nucleic acid-based biorecognition elements, against *Mycobacterium tuberculosis* ESAT-6/CFP-10 heterodimer protein by Capture Systematic Evolution of Ligands by EXponential enrichment (Capture SELEX). Considering the folding and stability of the ESAT-6/CFP-10 protein, we used capture-SELEX to isolate aptamers that can recognize the heterodimer target in solution in their native complexed state. To this end, we use the recombinantly expressed ESAT-6 and CFP-10 proteins to form the complex *in vitro*. During the selection, the single stranded DNA (ssDNA) library was first immobilized onto magnetic beads via hybridization with a partially complementary docking probe. Upon addition of the ESAT-6/CFP-10 heterodimer target, ssDNA sequences are displaced from the beads into the solution to enable binding to the target, followed by magnetic separation from the beads. Counter selection with human serum was also introduced to increase the specificity of selected aptamers and to ensure compatibility with detection in serum, a target matrix for non-sputum biosensing. High affinity aptamer sequences from enriched pools were analysed by Ion Torrent Next Generation Sequencing and bioinformatic analyses, and the binding properties of selected aptamers were characterized using enzyme-linked aptamer assays with monomeric and heterodimeric forms of the antigens. Overall, the strategies outlined highlight an approach of selecting aptamers tailored for biorecognition of ESAT-6/CFP-10 heterodimer complex towards non-sputum-based detection of tuberculosis.

**Keywords:** SELEX, tuberculosis, aptamers, biorecognition elements.

## Improving Cocaine Voltammetric Detection with Molecularly Imprinted Polymer Modified Sensor

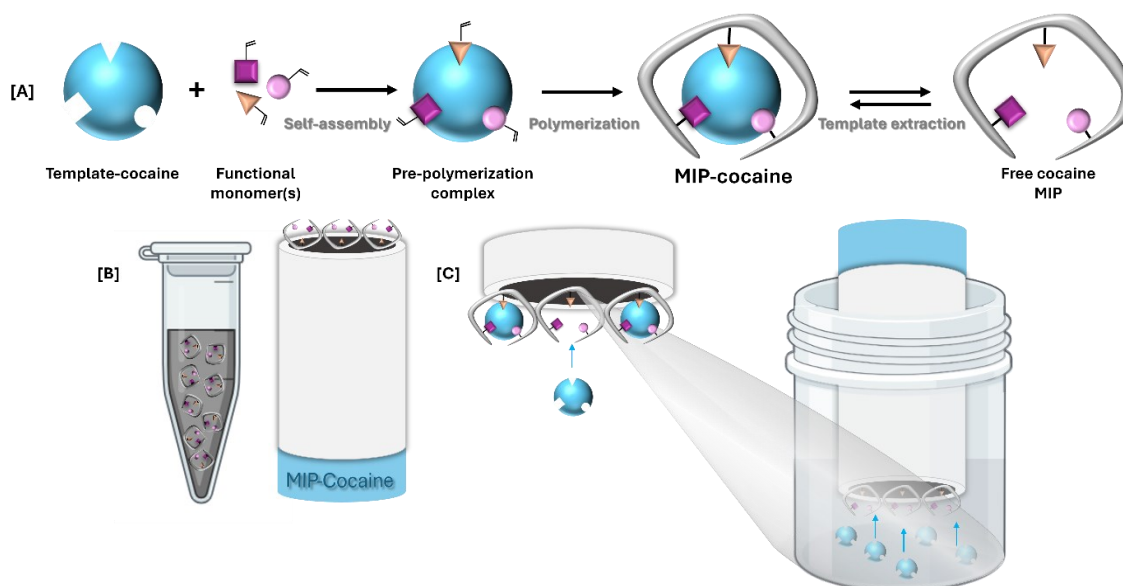
E. Rodríguez-Franch, X. Cetó, M. del Valle\*

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Increasing trafficking of illegal drugs and their precursors place law enforcement authorities in requirement of new analytical tools capable of achieving quicker analysis. A potential option for this might be portable and wireless devices based on the electrochemical technique that can reliably and sensitively detect these substances. The main challenges of the current in-situ methods used are the low accuracy for color tests and the high cost and limited portability for spectrometry/spectroscopic testing. In response to these challenges, BorderSens project aims to establish the basis for the development of a unique, portable and wireless device capable to quickly test different drugs, precursors and cutting agents, with improved sensitivity and specificity. This project combines the inherent advantages of electrochemical sensors, molecularly imprinted polymers (MIPs) and pattern recognition tools. Herein we present an overview of some of the achievements made so far in the detection of cocaine and some of their common cutting agents. Some of them, i.e. levamisole, show a suppressing effect on the signal of cocaine, which might cause false negative results. Two strategies were proposed to avoid this suppression and reveal the signal of cocaine on graphite epoxy composites (GEC) electrodes. First, the adjustment of the pH of the detection solution to pH 12. Besides, a second strategy involves the modification of cheap carbon electrodes with MIP of cocaine. These sensors achieved limits of detection of this drug in the  $\mu\text{M}$  range, showing the usefulness of the approach. Considering the inherent advantages of electrochemical methods, such as speed, simplicity and low cost, as well as the higher specificity of MIPs, the developed strategies offer a promising alternative for in situ screening of seized cocaine samples.

**Keywords:** illicit drugs, cocaine, levamisole, electrochemical sensors, molecularly imprinted polymers.



**Figure 1.** A) Scheme of MIP synthesis. B) Polymer integration onto the sensor surface. C) Direct electrochemical detection of the analyte.



## The Importance of Antibody Cross-Reactivity in Immunoanalytical Tools for Toxins

J. Reverté<sup>1,2</sup>, M. Alkassar<sup>1</sup>, M. Rambla-Alegre<sup>1</sup>, A. Sanchez-Henao<sup>1,3</sup>, M. Mandalakis<sup>4</sup>,  
P. Peristeraki<sup>4</sup>, F.X. Sureda<sup>2</sup>, J. Diogène<sup>1</sup>, M. Campàs<sup>1,\*</sup>

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<sup>2</sup> URV, C/ Sant Llorenç, 21, 43201 Reus, Spain.

<sup>3</sup> IUSA, University of las Palmas de Gran Canaria, 35416 Arucas, Spain.

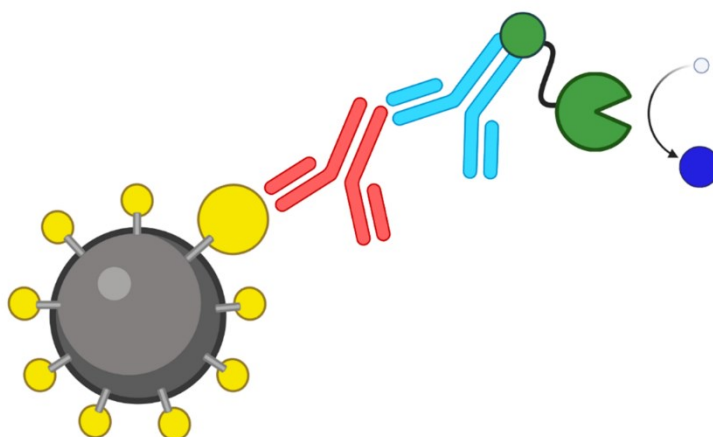
<sup>4</sup> HCMR, 71003 Heraklion, Greece.

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Antibodies are widely employed as biorecognition elements in the development of bioanalytical tools, such as immunoassays and immunosensors, due to their high specificity, which stems from their ability to selectively bind distinct structural regions on target analytes. While advantageous, this high specificity may present challenges in certain applications, particularly in food safety. In this context, it is crucial to detect not only the parent toxins in seafood but also their analogues, which can also pose a risk to human health. Therefore, evaluating the cross-reactivity of antibodies to toxin analogues is essential for validating bioanalytical tools and ensuring comprehensive threat assessment.

In this study, the impact of different antibody cross-reactivity toward structurally related toxin analogues on the performance of an immunoassay was investigated using tetrodotoxins (TTXs) as a model. A magnetic bead-based immunoassay was used to assess the affinity of the antibody for TTX analogues. The results demonstrated that the antibody exhibits lower affinity for TTX analogues compared to the parent TTX. However, since the antibody targets the same molecular site responsible for neurotoxicity, the detection capabilities from the immunoassay were found to be proportional to the toxicity of the analogues. These findings highlight the importance of considering the cross-reactivity when developing bioanalytical tools, not only when antibodies are used but also when other biorecognition elements are integrated into the analytical system.

**Keywords:** Cross-reactivity, antibody, immunoassay, tetrodotoxin, pufferfish, food safety.



**Figure 1.** Graphical schematization of the magnetic bead-based immunoassay used for assessing the affinity of an anti-tetrodotoxin (TTX) antibody towards TTX analogues.

## Neuroblastoma Cell-Based Tools and HPLC-FLP for the Detection of Paralytic Shellfish Toxins in Marine Pufferfish from the Spanish Mediterranean Coast

M. Alkassar<sup>1</sup>, À. Tudó<sup>2</sup>, M. Rambla-Alegre<sup>1</sup>, L. Ferreres<sup>1</sup>, J. Diogène<sup>1</sup>, F.X. Sureda<sup>2</sup>, M. Campàs<sup>1,\*</sup>

<sup>1</sup>IRTA, Ctra. Poble Nou del Delta, km. 5.5, 43540 La Ràpita (Tarragona), Spain.

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Pufferfishes, belonging to the order Tetraodontiformes, are some of the most toxic marine organisms, responsible for numerous poisoning incidents and some human fatalities due to their capability to accumulate potent neurotoxins such as tetrodotoxins (TTXs) and paralytic shellfish toxins (PSTs). In recent years, the geographical distribution of poisonous pufferfish has become more widespread. The potential spread of TTXs and PSTs through pufferfishes living in Mediterranean waters represents an emerging risk for European countries.

In this study, tissue extracts (muscle, skin, liver, intestinal tract and gonads) obtained from sixteen pufferfish specimens of the *Lagocephalus lagocephalus* and *Sphoeroides pachygaster* species, collected along the Spanish Mediterranean coast between 2014 and 2016, were analysed for the presence of voltage-gated sodium channel (VGSC) blockers using cell-based assay (CBA) and automated patch clamp (APC). No toxicity was observed in any of the *S. pachygaster* specimens, but toxicity was detected in the liver of most *L. lagocephalus* specimens.

Instrumental analysis of these specimens, as well as one *L. sceleratus* specimen, by high-performance liquid chromatography coupled to fluorescence detection (HPLC-FLD) confirmed the presence of PSTs only in *L. lagocephalus* specimens. This technique reported the presence of saxitoxin (STX) and decarbamoylsaxitoxin (dcSTX) in all positive samples, being dcSTX the major analogue. These results demonstrate the ability of this species to accumulate PSTs, being the first report of the presence of PSTs in Mediterranean *L. lagocephalus* specimens. Furthermore, the high PSTs contents in the five tissues of one *L. lagocephalus* specimen pointed the risk that the presence of this toxic fish in the Mediterranean Sea may represent for seafood safety and the economy of fishing and aquaculture industries.

**Keywords:** pufferfish, saxitoxin, tetrodotoxin, cell-based tools, HPLC-FLD.

## 3D-Printing Technology for Unconventionally Shaped Electrode's Fabrication

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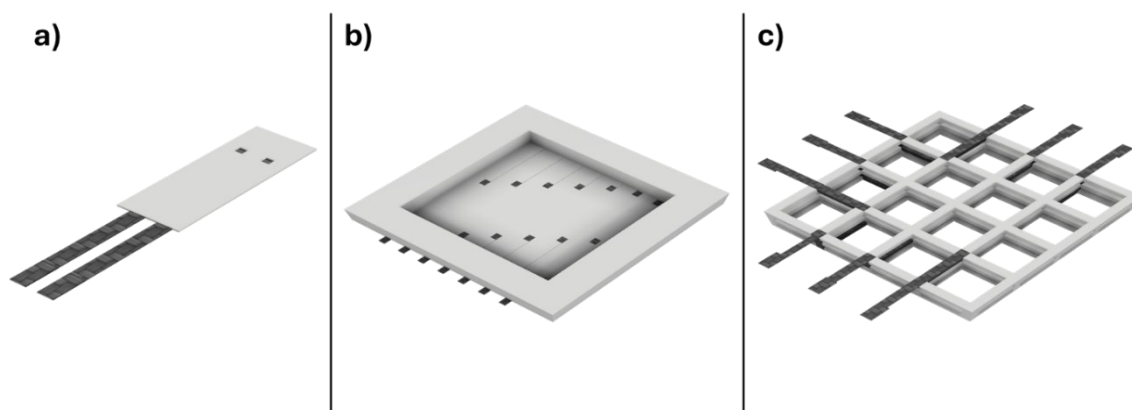
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Since the first electrodes were fabricated by using 3D-printing technology, innovations have focused on the usage of new materials, miniaturization and property improvement. However, 3D-printing has a great advantage over other fabrication technologies that is not sufficiently exploited. Compared to the rest, it does not require a substrate on top of which to produce the desired device. As its name suggests, it can print 3D objects, whereas other technologies can only work on 2D surfaces. This permits to create sensors with any shape.

The most widely used electrochemical sensor is the glass electrode pH sensor.  $H_3O^+$  concentration is a key parameter in a great deal of chemical reactions, resulting in a high demand for tracking technologies. To cover applications where conventional electrodes are inappropriate, new materials have been tested. Amongst those, iridium oxide-based (IrOx) sensors present some very interesting properties and has been selected as the sensing material to build this work's pH sensors.

The production of this sensor was done for a multiplate reactor. Interest was in monitoring pH inside a specific chamber without sample extraction. The first proposal was a thin electrode, using the basic configuration of two electrodes, placed one besides the other (**Figure 1A**). The sensing or working electrode is made of a conductive polymer modified with IrOx and the reference electrode is Ag/AgCl covered with a PVB<sub>NaCl</sub>-saturated membrane. Additionally, several sensors built into the plate that encloses the chamber (**Figure 1B**) and into the separator that fits inside the chamber (**Figure 1C**) were also fabricated. All have been characterized and compared to test the applicability of unconventional but multipurpose and functional shapes towards the fabrication of miniaturized sensors. Initial tests show a super-Nernstian response around -74.1 mV/pH, with stabilities over 15 days in a 3-11 pH range.

**Keywords:** 3D-printing, pH sensor, iridium oxide, device-miniaturization.



**Figure 1.** Three different shapes of 3D-printed pH sensors: **A)** conventional two-electrode potentiometric design; **B)** several sensors built into a reactor plate and **C)** several sensors built into a membrane separator.

## Porous Alumina-Based Biosensing Platforms for Early-Stage Infection Diagnosis

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To overcome the limitations of traditional diagnostic methods like PCR and ELISA, which require expensive and complex equipment <sup>[a]</sup>, we developed innovative biosensing platforms using porous anodic alumina (pAAO) on carbon screen-printed electrodes (CSPEs). These platforms are tailored for the sensitive, specific, and cost-effective detection of exosomes and miRNAs, crucial for early-stage disease diagnosis. Our first platform is designed to detect exosomes in serum with a theoretical limit of detection (LOD) as low as 100 exosomes per ml. It utilizes pAAO on CSPEs, capitalizing on the high surface area and customizable pore structures of pAAO <sup>[b]</sup> for effective exosome capture. The second platform focuses on miRNA detection in milk, boasting a theoretical LOD of 0.01 pM. This capability is achieved through structural and various target-specific optimizations of pAAO membranes on CSPEs for enhanced electrochemical sensing <sup>[c]</sup>.

These platforms not only enhance diagnostic sensitivity and specificity but also allow for comprehensive analysis of biomarkers in complex biological fluids. Preliminary studies have demonstrated the feasibility of using pAAO for efficient capture and sensitive detection of both exosomes and miRNAs. Designed to be portable and suitable for point-of-care testing, our biosensing platforms offer substantial social, economic, and environmental benefits. They provide a cost-effective solution that is expected to reduce healthcare costs and minimize environmental impact by lowering the need for chemical reagents.

In conclusion, our advanced nanostructured biosensing platforms represent a transformative advancement in early disease diagnosis. With the capability to detect minute quantities of biomarkers in diverse samples, these platforms promise to improve diagnostic accuracy and patient outcomes worldwide.

**Keywords:** exosomes, miRNAs, early disease diagnosis, porous anodic alumina, biosensing platforms, point-of-care testing.

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<sup>b</sup> G. Rajeev, et al. *Adv. Healthc. Mater.* **2018**, 7, 1700904.

<sup>c</sup> G. Rajeev, et al. *Front. Chem.* **2020**, 8, 1-11.

## Development of Electrochemical Sensors with 3D Printing Technology for the Analysis of Illicit Substances

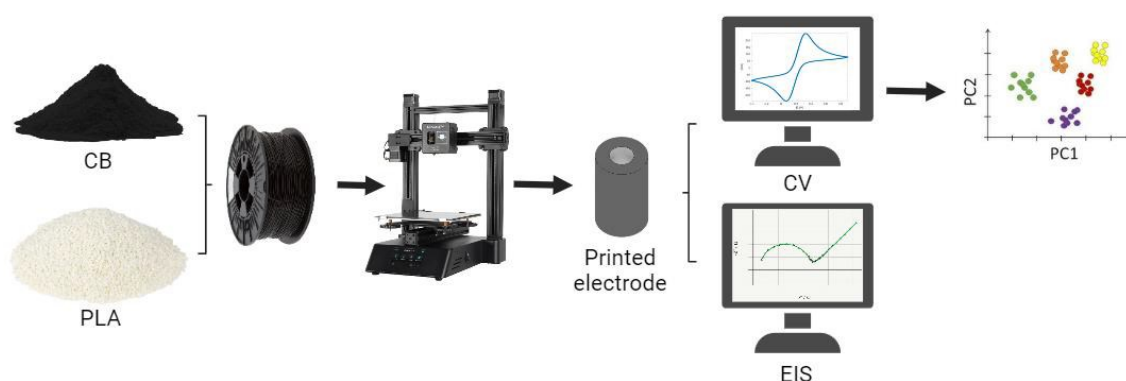
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The precise and rapid analysis of illicit substances is of vital importance for addressing social and public health issues. Efficient detection of these drugs can significantly improve law enforcement, public safety, and healthcare. On-site drug testing methods currently used show limited accuracy, while more advanced alternatives can be costly and require sophisticated equipment, which limits their accessibility and speed<sup>[a]</sup>. In this context, electrochemical sensors can provide an interesting alternative for the detection of illicit drugs and their precursors due to the inherent electrochemical behavior of most of them. More specifically, 3D printing technology, particularly fused deposition modeling (FDM), emerges as an innovative and accessible solution for creating electrochemical sensors. By using commercial filaments composed of polylactic acid (PLA) as an insulating material and carbon black (CB) as a conductive material, it is possible to manufacture electrochemical sensors with reduced cost and improved performance. In this study, the comprehensive development of voltammetric sensors towards the analysis of drugs is reported. Firstly, the design and optimization of the printing process were evaluated. Next, the use of an activation step of the sensor surface was also assessed using different approaches. To verify the performance of the sensors, their response against  $[\text{Fe}(\text{CN})_6]^{3/4-}$  was evaluated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Subsequently, a statistical analysis was conducted to ensure the reproducibility and lifetime of the devices. Finally, the developed sensors were applied for the analysis of different drugs such as cocaine or MDMA, between others. Under the optimized conditions, the developed sensors demonstrated a good response and discriminative potential, with excellent linearity at the  $\mu\text{M}$  level.

**Keywords:** 3D printing, fused deposition modeling, voltammetric sensors, illicit drugs



**Figure 1.** Schematic representation of the development of the 3D printed electrodes.

**References:**

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## Smart Analytical Microtechnology for Aquaculture Process Monitoring

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The rising global demand for environmental sustainability and enhanced industrial productivity has intensified the need for advanced instrumentation in aquatic process control. Current probes for continuous monitoring face significant limitations: they are expensive, require frequent maintenance and calibration, and are limited to measuring only a few specific parameters (pH, conductivity, dissolved oxygen, redox potential, and temperature). Critical parameters such as nitrogen derivatives (ammonium, nitrites, nitrates), phosphates, and microorganisms are still analyzed in laboratories, which is time-consuming and labor-intensive.

In this context, we propose the development of a smart multi-sensor system fabricated with cost-effective microelectronic technology combined with powerful data fusion tools like neural networks, to achieve a predictive system for on-line water quality monitoring. This system contains multi-sensor electrochemical sensors manufactured with microelectronic technology, being therefore robust, miniaturizable and scalable in its production. They detect indicators like pH, conductivity, temperature, chlorine, and ions (nitrate, phosphate, ammonium). Sensors are combined with local neuromorphic intelligence that, in addition to its predictive capabilities, allows to correct sensor drift, aging and interferences produced during their operation. These corrections minimize and even avoid need for calibration during long-term periods of continuous monitoring.

In this work we present the results of a probe containing a smart multisensor system developed in the frame of the IAQUA project funded by the AGAUR (Generalitat de Catalunya) and with IMB-CNM and IRTA-La Rapita partners. This probe will be deployed at aquaculture sites to assess fish conditions and improve decision making.

**Keywords:** smart multisensor, neuromorphic intelligence, aquaculture.

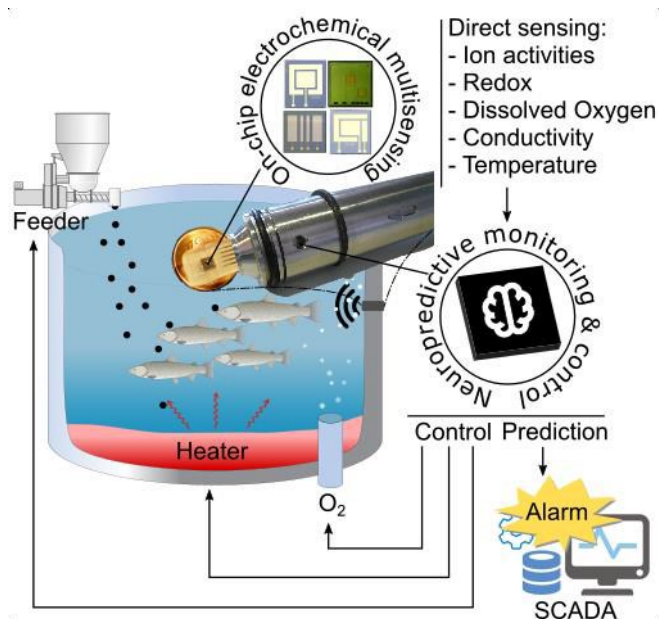


Figure 1. Scheme of sensors and neuromorphic systems measurements to control aquaculture processes.

## Portable PCR and Electrochemical Genosensing for the Rapid Screening Test Development to Detect Neonatal *Streptococcus agalactiae* Infections

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*Streptococcus agalactiae* (Group B Streptococcus, GBS) is the primary cause of neonatal infections, impacting pregnant mothers and, particularly, their offspring. This infection affects 18% of pregnant women globally, rising to 35% in certain regions. It leads to a range of diseases, including maternal infection, stillbirth, and early- and late-onset sepsis in newborns. Additionally, GBS may contribute to preterm delivery and hypoxic-ischemic encephalopathy. In 2020, an estimated 90,000 infant deaths were attributed to GBS, with nearly half occurring in Sub-Saharan Africa. To mitigate these alarming statistics, preventive antibiotics are proactively administered to the mother during delivery upon positive detection of GBS (typically around week 36 of pregnancy). This measure aims to prevent vertical transmission during childbirth and minimize risks to the baby. However, current detection methods rely on culturing techniques, which have long turnaround times and require sophisticated laboratory infrastructure and training. Diagnosing GBS in newborns is even more challenging, as it requires a minimum volume of blood and/or cerebrospinal fluid for successful culture, which can be difficult to obtain from these patients. Moreover, obtaining results can take several days. Given these limitations, this research project focuses on developing a point-of-care rapid screening test using a handheld thermocycler to perform double-tagging endpoint PCR. This involves conventional PCR with a novel thermostable DNA polymerase targeting a GBS-specific gene, screening various bacterial lysis methods, determining methodology specificity, and establishing the limit of detection. Our results demonstrate a simple, specific, and promising system for detecting the presence of GBS in patients. Further work will involve testing different matrices (blood, vaginorectal swabs) to explore the assay's sensitivity. The development of this device holds the potential to improve current protocols for GBS detection, allowing for rapid point-of-care screening both in expectant mothers and at the newborn's side. Such a breakthrough could significantly reduce the global burden of GBS disease, with a particularly positive impact in low-income countries where implementing microbiological cultures is challenging.

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## Portable Medical Device Based on Magneto Immunosensor for the Diagnosis of Celiac Disorder

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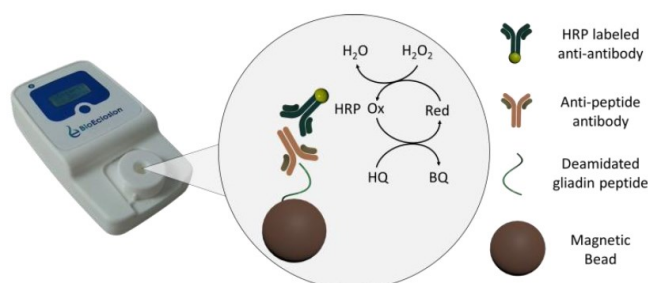
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Celiac disease (CD) is a chronic autoimmune disorder that affects individuals regardless of gender, age, or ethnicity, primarily arising from gluten intolerance in genetically predisposed individuals [a,b]. Although its prevalence impacts 1-2% of the global population, there has been a noticeable rise in reported cases over recent decades. The disparity between diagnosed and undiagnosed cases varies by country, with ratios ranging from 1 to 2 in Finland to 1 to 10 in the USA, Argentina, and Germany, suggesting a substantial percentage of cases remain undetected [c]. The main contributor to underdiagnosis is the variability of symptoms, which can mimic those of other diseases, ranging from small intestinal inflammation to diarrhea and other digestive disorders [a]. Current diagnostic methods, conducted in specialized laboratories, include serology, genotyping, and histology. Serology is considered the gold standard among these, despite its lower sensitivity, requiring further invasive tests for confirmation [d]. With the increase in cases, there is a critical need for rapid and accurate diagnostics, especially ones that simplify testing for community and primary care settings. To address this challenge, this work focuses on the study of novel biomarkers for CD based on synthetic deamidated-related peptides, enhancing the sensitivity of existing methods and integrating them into a novel magneto immunosensor. Derived from specific amino acid sequences of generic gliadin, these peptides exhibit a promising predictive value for CD detection. Therefore, when immobilized on magnetic particles, they can serve as distinctive biomarkers for the isolation of gliadin antibodies, and the detection can then be accomplished by amperometry using an enzyme-labeled antibody. The deamidated peptides are integrated on a device platform composed by two main components (**Figure 1**): A) Disposable cartridge, including i) the sample holder containing reagents (including magnetic particles modified with peptide and the labeled-antibody which binds specifically the patient antibodies in 15 minutes), and ii) the cartridge containing the microfluidic and the electrode in which the magnetic actuation is performed, while the excess of sample and reagents are removed. B) Digital reader which allows the electrochemical readout in less than one minute. The output of the device is a quantitative response. This test demonstrates significant potential due to its affordability, speed, sensitivity, minimal handling requirements, and suitability for ambulatory CD screening in primary care. The approach consolidates the competitive advantages of existing diagnostic tests, offering point-of-care convenience, rapidity, user-friendliness, quantifiability, and high predictive power.

**Keywords:** celiac disease, deamidated peptide, magnetic particles.



**Figure 1.** Schematic representation of the magneto electrochemical immunoassay.

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## Colorimetric and Electrochemical Enzymatic Biosensor for the Detection of Disinfectants in Dairy Products

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Cleaning and disinfection process is essential in the dairy industry to limit contamination by microorganisms and therefore to protect food safety. The most commonly used disinfectants are Quaternary Ammonium Compounds (QACs). Improper use (eg. overdose) or insufficient rinsing may lead to the persistence of residues on the surfaces; these residues could be transferred to dairy products and therefore could have undesirable effects on consumer's health. A European investigation in 2013 showed the contamination of dairy products by excessive concentrations of QACs, much higher than the Maximum Residue Limit (MRL = 100 µg/kg) <sup>[a]</sup>. The self-control methods currently used cannot reach the MRL. The objective of the SensoMilk project is to develop an innovative, sensitive, rapid and user-friendly biosensor, for on-site control.

Acetylcholinesterase enzyme (Ache) is used as a bioreceptor for the development of electrochemical and colorimetric biosensors. A recent study showed the possible detection of QACs in rinsing water, based on the inhibition of Ache activity <sup>[b]</sup>. Our colorimetric sensor is based on the classical Ellmann's method, which measures the Ache activity and so the inhibition by QACs <sup>[c]</sup>. Electrochemical biosensor is based on electron transfer following the oxidation of thiocholine, the product of the reaction. In the presence of QACs, the intensity of the oxidation peak will decrease.

The objectives of the microplate colorimetric assay were multiple. Firstly, the Michaelis-Menten constants (Km) in absence and in presence of QACs were calculated, which allowed concluding on the type of inhibition. Secondly, it allowed us to determine the optimal concentrations of Ache and substrate for the electrochemical biosensor' development. The microplate assay can detect QACs as low as 5000 µg/kg, which is not enough low (ie. 50 times higher than the MRL). Finally, a Lateral Flow Enzymatic Assay will be developed on the same principle. In parallel, we have worked on an electrochemical biosensor, based on the microencapsulation of Ache in tetraethylorthosilicate (TEOS) sol-gel on screen-printed carbon electrode. The enzymes were still accessible and active in the sol-gel film. Preliminary results that showed a detection capability of 100 µg/kg of QACs need to be confirmed. Moreover, other sol-gel will be tested and the performances will be compared.

**Keywords:** electrochemical, colorimetric, biosensor, acetylcholinesterase, quaternary ammonium compounds, food safety.

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<sup>c</sup> I. Cacciotti, et al., *Mater. Sci. Eng. C* **2020**, *111*, 110744.

# Electrochemical Determination of Emerging Contaminants Using Tailored Layer-by-Layer Modification of Screen-Printed Carbon Electrodes

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Emerging contaminants (ECs), including pharmaceuticals, industrial additives, and microplastics, pose a growing threat to environmental and human health. Their detection and quantification are crucial for understanding their impact and developing mitigation strategies. This study explores the development of a voltammetric method for EC detection using screen-printed carbon electrodes (SPCEs) modified with charged conductive polymer (nano)layers fabricated via customized layer-by-layer (LBL) strategies, based on dip coating, spin coating and drop casting. This work highlights the potential of LBL-modified SPCEs as a promising strategy for sensitive and efficient detection of emerging contaminants.

Surface characterization of the modified SPCEs confirmed successful modification through atomic force microscopy (AFM) and contact angle measurements. Electrochemical characterization demonstrated improved performance for detecting model EC analytes, including dopamine, paracetamol, and folic acid. The observed enhancement is attributed to electrostatic interactions between the immobilized polymeric network and the target analytes. Furthermore, the incorporation of magnetic cobalt ferrite nanoparticles into the measurement solution could improve repeatability, reproducibility, and sensitivity, potentially due to their role as diffusion enhancers.

**Keywords:** emerging contaminants, electrochemical detection, screen-printed electrodes, layer-by-layer assembly, voltammetry.

## Acknowledgements:

This work is financially supported by the project PID2022-136709OB-C22 funded by AEI/10.13039/501100011033/European Union NextGenerationEU/PRTR. J. Huang thanks the Institute of Water Research (IdRA) of the University of Barcelona and especially the China Scholarship Council's (CSC) financial support for the PhD fellowship.

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## Accurate and Fast Tetrodotoxin Detection by a Paper-Based Test Based on an Antibody-Aptamer Sandwich Assay

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Tetrodotoxin (TTX) is a small and highly toxic marine compound responsible for frequent intoxications globally. The toxin accumulates in marine organisms via the food chain and is commonly encountered in puffer fish and seafood. Cooking does not destroy TTX and after contaminated food ingestion, TTX blocks sodium voltage-gated channels and impairs nerve function. A dose of only 0.5 - 2 mg/kg body weight can cause severe symptoms in humans. Moreover, the lack of antidotes further emphasizes the need for the rapid and early detection of TTX in contaminated products in order to prevent poisoning incidents. However, the analysis of TTX is a challenging task, relying on expensive and complicated analytical techniques, which also require extensive sample preparation to overcome matrix effects. Lateral flow assays (LFAs) are fast and robust analytical tests in a paper-based, cost-efficient format. LFAs typically rely on a pair of bioreceptors for analyte detection. While one bioreceptor is immobilized on the nitrocellulose membrane, the second one is conjugated with a signal transducer which facilitates naked eye readout. Bioreceptors interact with the specific target analyte and can provide a signal-off or signal-on result. Competitive assays are based on a competition between sample target in solution and immobilized target for binding to the transducer bioreceptor and result in a signal-off event when target is present in sample. Sandwich assays, on the other hand, produce a signal-on result after binding of both bioreceptors to different regions of the target analyte, thus providing a more sensitive and robust detection system. Nonetheless, competitive assays are the most used systems for small molecule detection since it is very difficult to establish a sandwich format with such small targets. Aptamers are single stranded oligonucleotides with similar binding features as antibodies, with added advantages of higher stability, facile and more cost-effective production than antibodies. In recent years, aptamers are garnering increasing interest for the detection of small molecules. In our previous work we established a microplate antibody-aptamer sandwich-type assay for detection of TTX using an anti-TTX mouse IgG antibody and a newly selected TTX binding aptamer. In this work, the sandwich assay was implemented on an LFA strip to develop a test for the rapid and equipment-free detection of TTX. The test was performed in a single step, and it achieved a visual limit of detection of 0.3 ng/mL TTX in less than 20 minutes. The stability and specificity of the test were also demonstrated to be very high. Finally, its performance was validated with contaminated puffer fish samples. The test was able to detect TTX far below the safety limits of 2 mg TTX/kg fish tissue established in Japan, with absence of both matrix effects and cross-reactivity with other marine toxins. This TTX LFA test is the first sandwich-assay for TTX and significantly improves detection limits compared to current commercially available tests or other antibody-based competitive LFA tests published to date.

**Keywords:** puffer fish, lateral flow assay, small molecule detection, signal-on, food safety, aptamer, antibody.



**POSTER  
CONTRIBUTIONS**

## Integration of Molecularly Imprinted Polymers into Paper-Based Nucleic Acid Lateral Flow Platforms for Enhanced Multiplexed Point-of-Need Diagnostics

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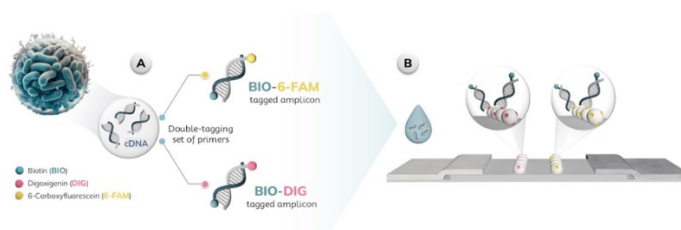
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Paper-based immunochromatography tests have become indispensable tools in point-of-need diagnostics, featuring portability, rapidity, and cost-effectiveness. Antibodies are commonly used as biorecognition elements in *in vitro* diagnostic tests due to their exceptional specificity and ease of chemical modifications. However, their inherent limitations, including susceptibility to chemical and physical denaturation, along with laborious and costly production processes, underscore the need for alternative recognition materials. In response to these challenges, molecularly imprinted polymers (MIPs) have gained considerable attention. MIPs display predetermined recognition sites for target molecules, acting as artificial receptors and offering distinct advantages that address key issues associated with antibodies [a]. Despite their biological counterparts, MIPs can be synthesized through an animal-free, large-scale process at a significantly lower cost. Moreover, their stability enables long-term storage at room temperature without compromising analytical performance.

This work presents, for the first time, the synthesis of MIPs specifically designed for digoxigenin (DIG) and 6-carboxyfluorescein (6-FAM), and their integration as distinct test lines into a multiplexed nucleic acid lateral flow (NALF) platform. Several components and the assay performance were optimized using the simultaneous detection of different double-tagged PCR amplicons from the waterborne pathogen *E. coli* as a model.

The integration of MIPs as recognition elements proves to be a cost-effective and robust alternative to affinity proteins, showcasing exceptional analytical features. The multiplexed assay demonstrates outstanding sensitivity and specificity, achieving a limit of detection of 0.11 pg and 0.38 pg for MIP-DIG and MIP-6FAM, respectively. This innovative approach not only enables the detection of a wide range of targets but also holds considerable promise for advancing paper-based diagnostic technologies.

**Keywords:** Molecularly imprinted polymers, multiplexed assay, point-of-need diagnostics, biomimetic receptors.



**Figure 1.** Schematic Representation of Multiplexed NALF: **A)** DNA extraction followed by PCR amplification using two different sets of primers. **B)** Samples are applied to the sample pad and migrate to the nitrocellulose membrane. On the membrane, MIP-DIG and MIP-6FAM selectively interact with the DIG and 6-FAM tags of the amplicons, respectively. Streptavidin-HRP is then added, enabling optical readout by interacting with the BIO-tag of the amplicons. Finally, the visual readout is achieved using TMB, along with the interpretation of the results.

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## Coupled Size-Exclusion and Electrocatalytic Activity of CuO QDs Functionalized Porous Silicon Electrodes Directly Used in Human Saliva

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The electrochemical determination of important biomarkers in biological samples is more often than not hampered by the presence of protein and other macromolecules which are known to foul the electrode surface in a way that prohibits their analytical determination. Usually, the alleviation of this problem entails the use of complex chemical (solvent precipitation, acid treatment, etc.) and/or physical (centrifugation, filtering, etc.) treatments. However, the use of these types of treatment, while efficient, negates the possibility of using the electrochemical biosensor directly at the point of care. Our work is based on the size-exclusion properties of carbonized porous silicon (pSi) transducers which rely on the adaptation of their pore size depending on the biofluid involved, thus acting as molecular sieves. By using quantum dots (QDs) of sub < 10 nm size we can functionalize the inner depth of the pores to enhance the electrocatalytic activity of the electrode depending on the targeted analyte. More specifically, our endeavors revolve around the use of copper (II) oxide (CuO) QDs anchored onto the pore structures for the determination of nitrate ( $\text{NO}_3^-$ ), and nitrite ( $\text{NO}_2^-$ ) ions in human saliva. The size of the QDs has been verified using dynamic light scattering (DLS) and high-resolution transmission electron microscopy (HR-TEM), while the QDs functionalized pSi demonstrated its electrocatalytic activity in human saliva via cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

**Keywords:** Size-exclusion, porous silicon electrodes, copper oxide quantum dots, saliva analysis.

## Laser-Induced Graphene Electrodes for Wearable Sweat Sensors

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In recent years, printable electronics have triggered a significant advancement in wearable technology within the realms of health monitoring and diagnostics. In this context, wearable sensors can offer non-invasive, uninterrupted, and real-time assessment of physiological processes. To illustrate, there has been a surge of efforts in wearable electronic devices designed to precisely measure vital signs like heart rate, body temperature, and blood pressure. In addition, as these sensors maintain direct contact with the biofluids, e.g., sweat, they offer the potential of personalized healthcare, with the capability to simultaneously and seamlessly detect numerous biomarkers in sweat. Most current methods for producing wearable sensors rely on inkjet-printed metallic electrodes, which limit the types of substrates due to the high sintering temperature required. An efficient alternative is laser-induced graphene (LIG) electrodes, which involve the direct conversion of carbon-containing precursors into graphene using a laser. This direct writing process allows for the customized fabrication of electrodes with complex designs, minimal chemical use, energy efficiency, and waste reduction. The simplicity and cost-effectiveness of the LIG process make it suitable for large-scale production without complex infrastructure, promoting broader adoption of environmentally friendly practices in electronic device manufacturing. Here, we developed LIG electrodes on polyimide for the detection of relevant biomarkers in sweat. Calcium ions ( $\text{Ca}^{2+}$ ), which play significant roles in conditions such as myeloma, cirrhosis, renal failure, and disorders related to acid-base balance, were targeted. To achieve this, LIG-based devices were modified with polymeric ion-selective membranes to detect  $\text{Ca}^{2+}$ , and next, their response were characterized. The integration of LIG-based sensors into wearable technology holds great promise for enhancing personalized healthcare, offering noninvasive and continuous monitoring of a wide range of physiological parameters and biochemical markers, potentially revolutionizing early diagnosis and management of various health conditions.

**Keywords:** Wearable, sweat sensor, laser-induced graphene, PVC membrane, calcium.

**Acknowledgements:** This work was supported by FAPESP (2023/07812-2).

## Inkjet-Printed Sensor Array for the Simultaneous Detection of Drugs of Abuse Adulterants

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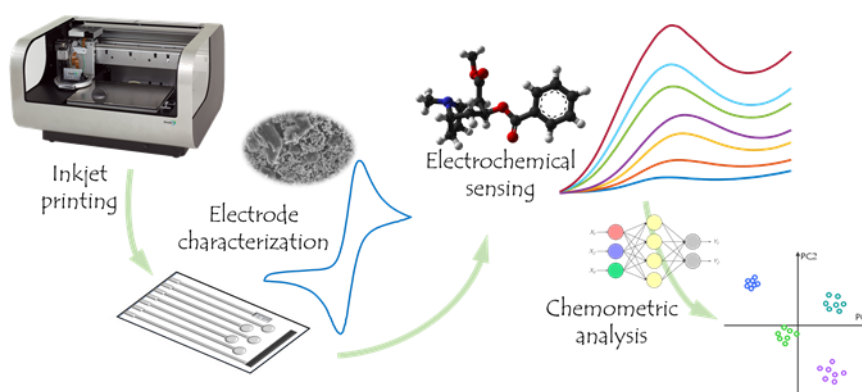
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To overcome the limitations of current decentralized drug testing devices, the development of compact sensors that are sufficiently sensitive and selective, inexpensive, and amenable for mass production is required. In this direction, electrochemical sensors can provide an interesting alternative for the detection of illicit drugs and their precursors due to the inherent electrochemical behavior of most of them <sup>[a,b]</sup>. Concretely, screen-printed electrodes have become a very well-established approach for the development of electrochemical-based portable devices over the last years <sup>[c]</sup>. However, despite the many benefits that screen printing provides, inkjet printing can outperform the former in terms of resolution due to the higher control of the deposited films, minimal waste generation or easier prototyping as no masks are required <sup>[d]</sup>. For these reasons, inkjet printing represents an alternative microfabrication method to conventional standard techniques for the development of flexible and low-cost devices.

Herein, the resolution and quantification of mixtures of different adulterants commonly present on drugs of abuse using an integrated inkjet-printed sensor array is evaluated. Specifically, benzocaine, paracetamol and phenacetin were considered. Firstly, the responses of the different sensors towards the different cutting agents were characterized using square wave voltammetry (SWV), showing a good performance with good linearity at the  $\mu\text{M}$  level. Subsequently, a quantitative model that allowed the identification and quantification of the individual substances from the overlapped voltammograms was built using artificial neural networks (ANNs) as the modelling tool. With this approach, quantification of the different drugs was achieved at the  $\mu\text{M}$  level, with a total normalized root mean square error (NRMSE) of 0.032 for the test subset.

**Keywords:** Electrochemical sensors, electronic tongues, artificial neural networks, cutting agents; illicit drugs.



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## The BLUESHELLFISH Project: Combination of Toxicological and Structural-Recognition Data for Ciguatera Risk Assessment

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Ciguatera poisoning (CP) is one of the most widespread non-bacterial seafood-borne illnesses globally, caused by the consumption of fish contaminated with ciguatoxins (CTXs). These potent marine neurotoxins are produced by microalgae of the genera *Gambierdiscus* and *Fukuyoa* and accumulate through marine food webs via a complex biomagnification process, ultimately reaching humans, thereby posing a significant threat to food safety and human health. Characterizing the risk of ciguatera in specific geographical regions remains a significant challenge due to the limited understanding of CTX congener diversity, their distribution across habitats and trophic levels, and their different toxicities. This challenge is particularly pronounced in the Indian Ocean, where few studies have focused on this region. To address these gaps, the BLUESHELLFISH project will employ a multidisciplinary approach, integrating toxicological and structural-recognition data, to evaluate the ciguatera risk in La Réunion Island.

To comprehensively assess the presence of CTXs in fish from the La Réunion Island region, multiple analytical techniques will be employed. Fish species known to be CTX carriers will be analyzed using both cell-based assays (CBAs) and immunoassays, targeting specific ciguatoxin series (CTX1B and CTX3C). The CBA offers a functional approach, providing a measure of the biological activity of the CTXs. By assessing the cellular response to the toxins, CBAs can detect not only known CTX congeners but also other structurally similar toxins that may not be identified by traditional chemical methods. This makes CBAs highly sensitive and suitable for detecting a broad spectrum of CTX analogues in complex samples. In parallel, the immunoassay will be used to specifically detect CTX congeners from the CTX1B and CTX3C series. Immunoassays are highly specific, relying on antibodies that bind selectively to the toxins of interest. This method allows for precise identification of particular toxin types, making it invaluable for confirming the presence of the most toxic and well-characterized CTX congeners in the fish samples. The combination of these two methods (toxicological and structural) provides a comprehensive analytical framework. While the CBA can detect the overall toxic potential of the fish sample, including novel or less characterized CTX congeners, the immunoassay offers high specificity in identifying key toxin variants. Together, these techniques will deliver a robust and reliable assessment of the CP risk in the fish population of La Réunion Island, advancing both our understanding of toxin profiles in the region and our ability to mitigate potential public health risks.

**Keywords:** Ciguatoxins, ciguatera poisoning, cell-based assay, immunoassay, food safety.

## Development of 3D-Printed Micropillar-Based Electrochemical Sensors

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The fabrication of sensors using 3D-printing, namely with an easy-to-use, reproducible, cost-effective and scalable technology, has been in the center of research interest in recent years. 3D-printing has been highlighted as an astounding approach due to its feasibility to fabricate sophisticated and complicated arrays of microstructured electrodes with high resolution and feature precision, having available a variety of new printing materials. Among the various 3D-printing techniques, stereolithography (SLA) has shown promise in constructing microstructured electrode surfaces, which are challenging or impossible to achieve with other conventional methods. This study focuses on fabricating electrochemical sensors based on micropillars of varying sizes by combining SLA 3D-printing with gold sputtering. These micropillar-based sensors exhibit increased electroactive surface area compared to planar sensors, resulting in improved analytical performance. The micropillar arrangement enhances the interaction of target molecules with the electrode surface, even at low concentrations, and facilitates both planar and radial diffusion of electroactive species. These features contribute to improve the sensitivity and, eventually, lower the limits of detection. Additionally, 3D-printing technology's attributes make it highly suitable for developing wearable sensors for health monitoring and disease diagnostics. The potential for low-cost mass production using 3D-printing technology is pivotal for translating research prototypes into commercial products. By integrating the micropillar array with the sensing layer, the developed sensors gain physical protection and resistance to mechanical stretch when worn, addressing issues related to friction-induced damage between the skin and sensor surface. In summary, micropillar-based sensors fabricated through SLA 3D-printing offer a promising solution to enhance sensor performance and durability, with significant implications for the advancement of wearable health monitoring devices.

**Keywords:** SLA 3D-printing, micropillar arrays, electrochemical sensors, wearable sensors.

## Microanalyzer for pH Determination in Wine

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Monitoring the winemaking process and the quality and safety of the final product requires the quantitative determination of a different key physico-chemical parameters including temperature, density, acidity, pH or other compounds which defines its organoleptic characteristics.

Climate changes has dramatically affected wine production. The main effects are related to an increasement in the grape sugar content, the earlier shift of the ripening period and the higher degradation of organic acids during the late stages of ripening. Acidity decrement represents a problem for wine industry as higher pH facilitates a more rapid rate of oxidation and it induces microbial contamination. Therefore, acidity is a critical parameter and primary driver of important management decisions to prevent contamination risks and the loss of sensory (organoleptic) attributes. Hence, control it in the wine production process is of great importance.

The International Organization of Vine and Wine (in French "Organisation Internationale de la Vigne et du Vin", OIV) has established a variety of analytical methods to determine the different key parameters which allows to control and optimize the whole wine production process. However, many of these procedures are tedious, need trained personal, require bulky instrumentation, with expensive and time-consuming maintenance protocols. So, alternative analytical tools which solve these inconveniencies are desirable.

In winemaking process, pH is crucial for microbial stability, colour, preservation, oxidation, tartrate stability, protein stability, and wine taste and astringency. The proper pH range for red wine is between 3.4 and 3.7 and around 3 for white wines.

In this work, the conjunction of miniaturization technologies and potentiometric determination were proposed. Miniaturization technologies has demonstrated its potential to develop microanalyzers for environmental and industrial purposes and allows integrating detectors (electrochemical, optical) and complex microfluidic structures combining the advantages provided by miniaturized systems (reduction of sample/reagent consumption, mass production and portability) and those provided by continuous flow analysis techniques (simplicity, versatility, connectivity, speed of analysis, automation of an analytical procedure, etc.). Regarding the detection system, Ion Selective Electrodes (ISEs) are widely used in this kind of microsystems due to its instrumental simplicity, low cost and easy to use.

In this work, different H<sup>+</sup>-selective membrane with different compositions have been prepared and evaluated in batch conditions to be used wines. After that, the pH selective membranes with better analytical characteristics have been integrated as detectors in microflow systems based on Cyclic Olefin Co-polymer (COC). Once optimized the chemical and hydrodynamic parameters of the fabricated microanalyzer, its performance in the determination of pH in wine samples were evaluated.

**Keywords:** pH, ion selective electrode (ISE), flow injection analysis (FIA), microsystem, cyclic olefin co-polymer (COC).

## Development of a Biosensor to Detect Cellular Heterogeneity During Fermentation

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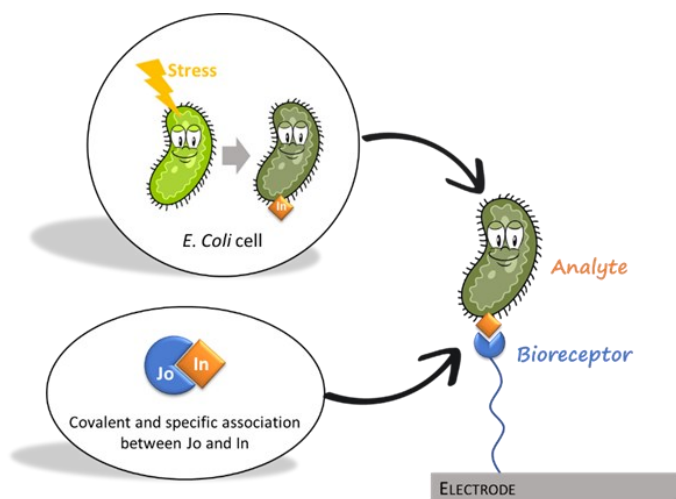
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Bio-based production of chemicals and fuels is important to develop a more sustainable society. For that, industrial processes based on microbial activities, such as fermentation, are becoming more widespread every year. In this kind of processes, microorganisms are used to produce a variety of different chemicals such as amino acids, vitamins, antibiotics, biofuels, etc. However, the scale-up of many biotechnological processes is still a challenge, and one of the reasons for this is the appearance of low-producing or non-producing cells in the fermentation reactors, which can reduce product yield and quality. This is due to the heterogeneity among microorganisms of the same species, which can appear in response to changes in environmental conditions (alterations of pressure, osmotic pressure, substrate availability oxygen concentration, etc.).

To avoid limitations in industrial-scale biomanufacturing of chemicals and fuels, it is essential to detect and prevent heterogeneity in microbial populations employed in fermentation reactors. This requires a better detection capacity of divergent cell phenotypes as early as possible. In this context, biosensors appear attractive devices for rapid and specific analysis of cell heterogeneity.

In this project, we propose to develop an electrochemical biosensor for the specific detection of diverging cell phenotypes in fermentation reactors, based on the specific association between two proteins, called “Jo” and “In”. Jo and In are able to bind to each other spontaneously in a very highly specific way, so that they can be employed to develop affinity biosensors. The idea is to immobilise one of these proteins (Jo) at the surface of an electrode, and conditionally express the other one (In) at the surface of divergent cells. The association between these two proteins, and consequently the presence of divergent cells, will be detected thanks to a change in the electrical signal of the sensor.

**Keywords:** Electrochemical biosensor, bioelectrochemistry, biotechnology.



**Figure 1.** Principle of the proposed electrochemical affinity biosensor to detect divergent cells. The Jo protein (in blue) is first immobilised at the electrode surface. *E. coli* cells (green, used as a model organism) exposed to stress express the In protein at their surface. These cells are then captured at the electrode by the formation of a covalent bond between Jo and In.

## Towards the Industrialization of Disposable Biomedical Devices for At-Home Use

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Modern (bio)analytical chemistry faces the important challenge of developing and validating new instrumentation methods that can work by providing truthful, real-time, in-situ and accurate information on molecules present at low sample volumes. In addition, in many areas it is desirable to bring the analysis closer to the user, avoiding the use of specialized laboratories and sophisticated and expensive instruments. This clearly implies a necessary simplification and automation of the (bio)analytical process, reducing sample volume and reagent consumption and minimizing manual intervention. It is therefore obvious to focus efforts on the miniaturization of instrumentation.

In the biomedical field, an important objective to be achieved is to provide patients with better follow-up of their diseases through the monitoring of their biomarkers and greater empowerment by home disposable medical sensor devices that do not require the presence of qualified technicians for their use, that is, Point-of-Care (POC) technology.

In this research, a versatile sensor platform is designed, developed and evaluated using Cyclic Olefin Copolymer (COC) and its manufacturing technology that offer great versatility for the development of this type of instrumentation. They involve a simple multilayer manufacturing process, compatible with electronic technology, thus permitting the inexpensive integration of sensors and electronic components in a cost-effective manner, which allows the production of disposable, biocompatible and transparent devices.

In this study, the device developed integrates two ammonium ion, a reference biomarker of some metabolic diseases, selective electrodes, one acting as an indicator electrode and the other as a reference, with the aim of quantifying the concentration of this ion in blood by means of potentiometric measurements. Additionally, a module integrating a gas-diffusion membrane is added to eliminate possible interferences contained in the samples to be analyzed.

The objective of this research is focused on the optimization of the chemical parameters involved in the ammonium measurement process through a disposable home use biomedical potentiometric device for its future industrialization and commercialization, and suitable for the measurement of biomarkers of other diseases. Among these optimizations are: selection of the polymer membrane composition, evaluation of the ionophore stability, selection of the diffusion membrane, evaluation of the analyte diffusion rate, among others.

The results obtained throughout the study reflect that a future industrialization of these devices is possible and that they are suitable for their use as disposable biomedical devices for home use to monitor different diseases by the user.

**Keywords:** Point-of-care (POC), potentiometry, ammonium ion, biomedical analyzers.

**Acknowledgements:** Authors are grateful for the financial support to the Spanish Ministerio de Economía, Industria y Competitividad and Ministerio de Ciencia e Innovación through the projects PID2020-117216RB-100, to Catalan government through the project 2021SGR00124 and to the Fundación PKU y otros trastornos metabólicos hereditarios.

## Disposable Device for At-Home Alanine Blood Monitorization for Controlling Mitochondrial Diseases

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Among the variety of rare inherited metabolic disorders that exist, we find the so-called mitochondrial diseases (MS). MS is a heterogeneous group of diseases caused by genetic mutations that prevent the proper functioning of mitochondria. These have in common that they are caused by a deficiency in ATP biosynthesis, as a result of defects in the functioning of the oxidative phosphorylation system, responsible for generating the energy necessary for the development and correct function of organs and systems, mainly heart, muscles, and brain. It is a group of chronic diseases with high variability and in many cases disabling. Most of them begin in childhood, with a prevalence of 1 in 5000 newborns, although they may appear, less frequently, in adulthood.

Its diagnosis focuses on biochemical and genetic tests, including the monitoring of one of its biomarkers in blood, alanine.

Currently, the measurement of this amino acid is carried out exclusively in specialized centers, a fact that has led to the search and development of reliable tools that meet clinical needs with the aim of improving the patient's life quality. Thus, miniaturized medical care and diagnostic devices, Point-of-Care (POC) technology, with low reagent and sample consumption, minimum analysis time, portable, automatic, highly robust, and low cost are required, which allow the rapid determination of alanine by the patients themselves, without the need for the intervention of qualified personnel.

In this research, the development, adaptation, and optimization of a versatile microfluidic platform has been carried out, using cyclic olefin copolymer technology, capable of executing potentiometric measurements in situ. These measurements are carried out by integrating two ammonium ion selective electrodes into the device, one acting as a reference electrode and the other as an indicator. A gaseous diffusion membrane is placed on the latter, which allows the concentration of the analyte of interest in the blood to be measured, indirectly and exclusively, by degrading it into ammonium by a specific enzyme. Throughout the study, the optimization of instrumental, chemical, and kinetic parameters such as: pH, analysis time, diffusion membrane, among others, is carried out. The experimental evidence obtained allows us to ensure that the Point-of-Care device developed will be able to measure alanine concentrations in blood for home control of mitochondrial diseases.

**Keywords:** Point-of-care (POC), potentiometry, alanine, ammonium ion, enzymatic degradation.

**Acknowledgements:** Authors are grateful for the financial support to the Spanish Ministerio de Economía, Industria y Competitividad and Ministerio de Ciencia e Innovación through the projects PID2020-117216RB-100, to Catalan government through the project 2021SGR00124 and to the Fundación PKU y otros trastornos metabólicos hereditarios.

## Porous Silicon-Based Sensor to Detect and Monitor Biofilm Growth

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Biofilms are 3D architectural communities of microorganisms that create favorable environments allowing for the attachment, colonization and population growth on both biotic and abiotic surfaces. These films are self-produced by the resident organisms, and their properties make them very difficult to remove both physically and chemically which causes numerous issues. Thus, many industries are affected by biofilms. Contamination of potable water, biocorrosion of naval vessels' hulls and build-up of dental plaque are to name a few issues where biofilms have been identified as the root cause. However, it is in the clinic where the negative impact on human health is most obvious as they are implicated in medical device failure and 85% of all chronic infections <sup>[a-c]</sup>. Along with protective and shielding properties from harsh external environments, biofilms also facilitate horizontal gene transfer which cumulates into developing antimicrobial resistance <sup>[e]</sup>.

Biofilm formation is a dynamic process and once mature, any intervention is often too late to make any successful attempt at removing it. There is a lack of detection methods that can identify the early stages of biofilm development. Most currently employed techniques require the operation of significant laboratory equipment and/or have long turnaround times <sup>[f]</sup>. Successful techniques for detecting early biofilm development would allow for clinicians to make earlier and thus more successful interventions.

The heterogenous nature of biofilms presents unique challenges in the development of reliable and robust sensing techniques. Physical, chemical and genotypical characteristics can vary both within biofilms and from biofilm to biofilm even when comparing samples of the same bacterial species. The invasiveness of any potential detection methods also need great consideration as an invasive technique in itself can change the properties of the biofilm.

Electrochemistry offers a non-invasive novel approach to monitor biofilm development with a quick turnaround of results. In this context, particular attention should be given to the design of the working electrode. Here we choose to work with an electrode that also acts as a substrate which physicochemical properties can be fine-tuned to control biofilm growth. Our previous research underpins porous silicon (pSi) as ideal candidate to suit the requirements of such platform. On the one hand, the possibility to tune pSi morphological features and surface chemistry has been demonstrated to be key to use this material as a scaffold for cell culture. This opens up new avenues to explore pSi suitability to support biofilm formation. On the other hand, we have reported a simple method to carbon-stabilize pSi, turning it into an excellent electrochemical transducer <sup>[g]</sup>. Here we present preliminary results of the use of various pSi structures as a substrate to grow *Staphylococcus epidermidis* biofilms, to further explore their potential as electrochemical sensors able to monitor biofilm growth in real-time.

**Keywords:** Biosensor, biofilm, electrochemistry.

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## Visual Test for the Detection of $\gamma$ -Glutamyl Transpeptidase in Calf Serum

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$\gamma$ -Glutamyl transpeptidase (GGT) is an enzyme involved in amino acid transport and produced in the mammary gland ducts during colostrogenesis. For this reason, its concentration in bovine colostrum is higher than in milk. When calves are fed with colostrum, GGT is absorbed in the small intestine via the same non-selective passage used by IgG. After absorption, GGT can be found in calf serum at concentrations 60-160 times higher than in adult cattle. Since a positive correlation has been observed between IgG and GGT, this enzyme has been identified as a potential biomarker to know the immunological status of calves at arrival at the rearing facilities.

The purpose of this work has been to develop a simple bioanalytical tool to detect GGT in calf serum, which could be applied by producers to design vaccination protocols in function of the immune status of the calves. Therefore, a portable and user-friendly visual test has been developed. The principle behind the visual test is the GGT enzyme reaction, where the GGT catalyses the transfer of amino acids between two peptides, L- $\gamma$ -glutamyl- $\gamma$ -(3-carboxy-4-nitroanilide) and glycylglycine, which results in L- $\gamma$ -glutamyl-glycylglycine and 5-amino-2-nitrobenzoate, the latter providing a change in the absorbance. In this work, L- $\gamma$ -glutamyl- $\gamma$ -(3-carboxy-4-nitroanilide) and glycylglycine have been co-immobilised on membranes by adsorption and subsequently exposed to GGT to detect its activity with the naked eye. Different types of membranes (e.g. nitrocellulose, polyester, cotton, glass fibres and, if necessary, combinations of these) have been tested to find the materials that provide appropriate high surface-to-volume ratio, stability and porosity, with the final purpose to get enough intensity and uniformity of colour. The yellow coloration observed with the naked eye was proportional to the GGT activity. The visual limit of detection (LOD), working range and sensitivity have been evaluated.

Additionally, the use of a simple blood separation system to obtain high-quality serum from blood obtained by pinching the ears of calves has been evaluated. This system could facilitate the work at the farm as it would not be necessary to collect blood from the jugular vein.

Once optimised and validated, this bioanalytical tool will increase the efficacy of the vaccines, increase the health and welfare of the animals, reduce antimicrobial use and resistances, and make the sector more professional at the time to make decisions.

**Keywords:**  $\gamma$ -glutamyl transpeptidase (GGT), membrane, calf, serum, immune status.



## Characterization of Microfluidic Platforms for their Application in Assisted Reproduction Processes

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The efficiency of assisted reproduction processes tends to be relatively low, despite the continuous and significant advances achieved so far. Some of the causes, particularly in oocyte maturation and in vitro fertilization, are due to the high level of human manipulation required at the different stages (such as the handling and transfer of reproductive cells, as well as changes in the culture medium). Manipulation of gametes is essential and a challenging task that depends on the skills of the operator. However, the oocyte fertilization and embryo culture steps in these reproductive techniques, such as in vitro fertilization, require multiple cycles of oocyte rinsing and handling during medium renewals or isolation of individual oocytes. Any mistake can lead to gamete or embryo degradation/stress. To try to overcome these drawbacks, it is proposed to automate this procedure.

In the present work, different studies are presented dedicated to evaluating different key aspects in the automation process of assisted reproduction techniques, taking as a starting point, different developed biocompatible microfluidic platforms.

First, the interaction between the biological materials and the surfaces of the microfluidic platforms that will be used in the assisted reproduction process will be studied. The absorption/adsorption capacity of the materials will be evaluated and tools will be explored to passivate or block these retention points that can modify the compositions of the culture media or can retain cells in an uncontrolled or unwanted way or even damage cells.

Secondly, different strategies will be studied to avoid the formation of bubbles during assisted reproduction processes and, in case of their appearance, to eliminate them in a simple and efficient way. Finally, the fluid dynamics of a microfluidic structure designed to carry out in vitro fertilization will be studied, with the intention of visualizing the possibilities offered by the microfluidic platform to automate the different stages such as medium renewal, introduction of gametes, fertilization, cell growth, etc.

**Keywords:** Assisted reproduction processes, In vitro fertilization, microfluidic platforms, automation, gametes.

**Acknowledgements:** The authors would like to thank the financial support from Spanish Ministry of Science and Innovation through the project PID2020-117216RB-I00 and Catalan government through the project 2021SGR00124.

## Cadmium Monitoring Using Automated Microanalyzers in the Hydrometallurgical Production of Zinc

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Zinc is a malleable metal with an excellent resistance to abrasion and corrosion. For these reasons, it is widely used in different industries such as automotive, construction and maritime transport. However, zinc is found in nature combined with other metals in zinc ores. In the hydrometallurgical production of zinc, to ensure high efficiency and performance of the process, the other metals present in the process that act as interferences in the final electrolysis stage must be monitored and efficiently eliminated. This is important to reduce the costs associated with their elimination and not compromise the quality of the final product. Cadmium is one of these interferences.

In this work an automated and miniaturized analytical system including a multicommutation subsystem to automate the analyzer calibration and sample analysis, a separation module based on an ion exchange microcolumn to eliminate interferences, and a microfluidic platform integrating mixing microstructures, reaction coils and the detection cell for the spectrophotometric determination of cadmium ion, is presented.

Different instrumental, hydrodynamic and chemical variables were optimized in order to obtain a linear range from 0.08 to 2 mg L<sup>-1</sup> of Cd (II), with a limit of detection of 0.02 mg L<sup>-1</sup> Cd (II). Synthetic samples simulating the real composition of the electrolytic solution in the cadmium elimination stage were analyzed providing very promising results.

**Keywords:** Cadmium, microanalyzers, automation, spectrophotometry, hydrometallurgical production of zinc.

**Acknowledgements:** The authors would like to thank the financial support from Spanish Ministry of Science and Innovation through the project PID2020-117216RB-I00 and Catalan government through the project 2021SGR00124. The authors also acknowledge financial support from Met-Mex Peñoles S.A. de C.V. and the Tecnológico Nacional de México.

## Cobalt Monitoring in the Hydrometallurgical Production of Zinc Using an Automated Spectrophotometric Microanalyzer

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Zinc is a nonferrous metal with interesting properties like its excellent malleability, and abrasive and anti-corrosion resistance. For all these reasons, zinc has been widely employed in automotive, construction and shipping industries. Zinc is found in the nature in ores combined with other metals, such as copper, cadmium and cobalt. The production of zinc involves extraction of pure metallic zinc from these raw minerals using the zinc hydrometallurgical process. It is composed by different stages in which the monitoring at real time of the concentration of the different metallic ions normally presents as impurities in the process solutions is required. This analytical control allows optimizing the yield of the hydrometallurgical process and the purity of the final product.

In this work, a continuous-flow spectrophotometric analytical microsystem is presented to monitor the concentration of cobalt present in zinc hydrometallurgical plants during the process of purification. The system includes a multicommutation subsystem to automate the analyzer calibration and sample analysis, and a microfluidic platform integrating mixing microstructures, reaction coils and the detection cell for the spectrophotometric determination of cobalt ion. 3-Hydroxy-4-nitroso-2,7-naphthalenedisulfonic acid disodium salt (NRS) was used as selective colorimetric reagent.

Different instrumental, hydrodynamic and chemical variables were optimized in order to obtain a linear range without interference from sample matrix between 0.2 and 1.5 mg L<sup>-1</sup> of Co (II), with a limit of detection of 0.07 mg L<sup>-1</sup> Co (II). Sampling rate achieved was 36 samples h<sup>-1</sup> with a minimum waste generation of 3 mL per analysis. Synthetic samples simulating the real composition of the electrolytic solution in the stage of cobalt elimination were analyzed providing very promising results.

**Keywords:** Cobalt, microanalyzers, automation, spectrophotometry, hydrometallurgical production of zinc.

**Acknowledgements:** The authors would like to thank the financial support from Spanish Ministry of Science and Innovation through the project PID2020-117216RB-I00 and Catalan government through the project 2021SGR00124. The authors also acknowledge financial support from Met-Mex Peñoles S.A. de C.V. and the Tecnológico Nacional de México.

## Evaluating the Capability of Pentamethincyanines as Bactericides

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Increasing concern of antibiotic resistance has promoted to explore innovative treatments to inhibit pathogenic microorganisms. In this regard, photodynamic therapy (PDT) is a promising method. Photosensitizers are known to be capable of effectively inactivating gram-positive when at the same time are harmless towards gram-negative bacteria, due to fundamental differences in cell wall structures. Nevertheless, the development of cationic photosensitizers, among them the pentamethincyanines, demonstrated an effective inactivation of bacteria without additional pretreatment of the cell wall. Due to its cationic nature they show unique chemical properties, which can be used against *Escherichia coli* (*E. coli*) through radiation and induced generation of singlet oxygen.

In this study, *E. coli* cultures of specific concentration have been mixed with pentamethine cyanine dyes (synthesised in the Sensors and Biosensors Group) and exposed to the radiation of a superLED emitting at 670 nm for the evaluation of their antibacterial properties by measuring the % of decrease of bacterial growth for possible water treatment.

The characterization of the generation of singlet oxygen has been carried out by means of UV-Vis spectrophotometry and a new developed miniaturized optical device with the markers 1,3-diphenylisobenzofuran (DPBF) and 9,10-Anthracenediyl-bis(methylene)-dimalonic acid (ABDA), in comparison with the reference methylene blue.

**Keywords:** Pentamethine cyanines, photodynamic therapy, bactericide, *E. Coli*.

**Acknowledgements:** Authors are grateful for the financial support to the Spanish Ministerio de Economía, Industria y Competitividad and Ministerio de Ciencia e Innovación through the project PID2020-117216RB-100 and to Catalan government through the project 2021SGR00124.

## Traffic Light-Based Point-of-Care Test for the Rapid Stratification of Fever Syndromes

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Recent evidence highlights the significance of sTREM-1 and Ang-2 as quantitative novel biomarkers for assessing disease severity, treatment response, and outcomes in various conditions, including malaria, pneumonia, COVID-19, among others fever syndromes. Elevated sTREM-1 levels have shown strong correlations with disease severity, multiple organ dysfunction, and mortality.

To aid in risk stratification, patients in this study were classified into three groups based on their sTREM-1 levels using the WHO-proposed traffic light color system.

A threshold value of 239 pg/mL represented the "green light," indicating low risk, while levels exceeding 629 pg/mL were designated as "red light," signifying an urgent need for admission. An intermediate "yellow light" indicated further monitoring. In this work, we present a rapid test integrating magnetic separation and electrochemical biosensing on a portable device operated by batteries. The laboratory prototype comprises two components: (1) a disposable cartridge containing magnetic particles conjugated with antibodies specific to the biomarker of interest, and (2) a digital reader equipped with an interface for quantitative electrochemical readout. The cartridge's microfluidic system facilitates magnetic actuation, while excess sample and reagents are removed. Within less than one minute, the digital reader provides quantitative readout of the biomarker levels in pg/mL, which is then displayed on the device's screen and transmitted to the accompanying App via Bluetooth.

The device's performance in classifying sTREM-1 levels is presented, demonstrating excellent readout results with a short 15-minute incubation step and a swift 2-minute readout process. This innovative point-of-care test holds great promise for aiding clinicians in rapid risk stratification and timely decision-making, potentially enhancing child survival outcomes and improving patient management in a variety of fever syndromes and specific diseases.

**Keywords:** Disease severity, point-of-care test, sTREM-1.

## The Evolving Landscape of Electroanalysis at the University of Barcelona

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The Electroanalysis Group at the University of Barcelona, established in the 1980s by Prof. Enric Casassas, has continuously adapted its research focus to reflect advancements in analytical chemistry. We have transitioned from traditional mercury electrodes to modern, modified screen-printed electrodes (SPEs) and sensor arrays. Our approach has shifted from classical electrochemical modelling to the implementation of chemometrics for electroanalytical data analysis. Additionally, we have incorporated amperometric detection into flow systems such as liquid chromatography (HPLC).

Beyond the initial focus on heavy metals, our research interests have broadened to encompass biomolecules, organic contaminants, and food analysis. This poster highlights our current research directions and future aspirations. We aim to develop sensor systems and data analysis strategies that provide valuable on-site alternatives to more expensive, less portable techniques like mass spectrometry (MS) and inductively coupled plasma (ICP).

Our current research areas include:

- Development of electrochemical sensors using commercially available SPEs, modified with various nanomaterials (reduced graphene oxide, carbon nanofibers, phosphorene, bismuthene), nanoparticles (primarily noble metal and metal oxide nanoparticles, and electrically charged polymers).
- Integration of electrochemical sensors into sensor arrays and electronic tongues (ET) for enhanced analytical capabilities.
- Utilizing HPLC and ET for the characterization, classification, and authentication of food products, with a focus on detecting adulteration.
- Employing spectroelectrochemistry (SEC) to create sensing systems that combine both optical and electrochemical measurements. In this sense, magnetic nanoparticles are being used for analyte pre-concentration and improved mass transport.
- Innovative 3D-printing technology for the design of customized electrodes and measuring cells.
- Development of advanced chemometric strategies for data acquisition, integration, and analysis.

**Keywords:** Electroanalysis, sensor development, screen-printed electrodes, spectroelectrochemistry, HPLC, food analysis, 3D-printing.

**Acknowledgements:** This work is financially supported by the project PID2022-136709OB-C22 funded by AEI/10.13039/501100011033/European Union NextGenerationEU/PRTR. J. Huang and J. Pagès-Rebull thank the Institute of Water Research (IdRA) of the University of Barcelona for the acquisition of consumables. J. Huang especially acknowledges the China Scholarship Council's (CSC) financial support for the PhD fellowship. J. Pagès-Rebull thanks for his PhD Grant reference PREP2022-000174 funded by MICIU/AEI/ 10.13039/501100011033 and by the European Social Fund (ESF+).

## Detection of Saxitoxin Using an Immunodetection Tool Based on Magnetic Beads

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Globally, 35% of events associated with shellfish poisoning from 1985 to 2018 are attributed to paralytic shellfish poisoning, primarily caused by Saxitoxin (STX). STX is a highly toxic neurotoxin that can block nerve impulse transmission and cause death by respiratory arrest, posing significant risks to environmental and human health.

Traditional detection methods are costly and include the mouse bioassay, which faces ethical scrutiny. Thus, new detection approaches are necessary. This study introduces an immunoassay where saxitoxin is immobilized on magnetic beads. Detection is achieved through the competition between free STX in the sample and immobilized STX for the binding site of an anti-STX antibody. A secondary antibody labeled with horseradish peroxidase is used to reveal the signal. The developed colorimetric immunoassay demonstrates a dose-dependent response with an IC<sub>50</sub> of 10 ng\*ml<sup>-1</sup> and a detection limit of 40 µg\*kg<sup>-1</sup>. It shows minimal matrix effects with a loading capacity of 250 mg of shellfish meat\*ml<sup>-1</sup>, enabling the detection of saxitoxin in shellfish meat samples.

**Keywords:** Saxitoxin, immunodetection, magnetic beads.

# Biochar Based Sustainable Electrochemical Screen-Printed Carbon Sensors for Environmental Analysis: A Perspective Approach

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Since biomass is widely available, has abundant reserves, is renewable, and has low production costs, research on its carbonized form, biochar, is currently of great significance [a,b]. Among them, sensors made from biocarbon materials have received widespread attention and improvement. We propose a novel surface functionalization strategy for sensor modification by converting rapeseed biomass into conductive carbon materials using drop casting for modification of screen-printed carbon electrodes (SPCE). Bare SPCE were modified by drop-casting, using different amounts of biochar obtained under different pyrolysis temperatures. Electrochemical characterization was based on the voltammetric method of redox pair probe, amperometry, and electrochemical impedance spectroscopy (EIS) research. The obtained electrochemical sensor, as is based on modified screen-printed carbon electrode with a drop-casting solution containing biochar material, has convenient sustainable characteristics, being as rapid as the bare SPCE. Furthermore, small sample instant detection can be applied to multiple purposes of environmental analysis detection [c].

**Keywords:** Screen-printed electrode, biomass/biochar electrochemical sensor, environmental analysis.

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## In-Vitro Diagnostic Test Based on Exosomes for Early Diagnosis of Alzheimer's Disease and Risk Stratification of Patients

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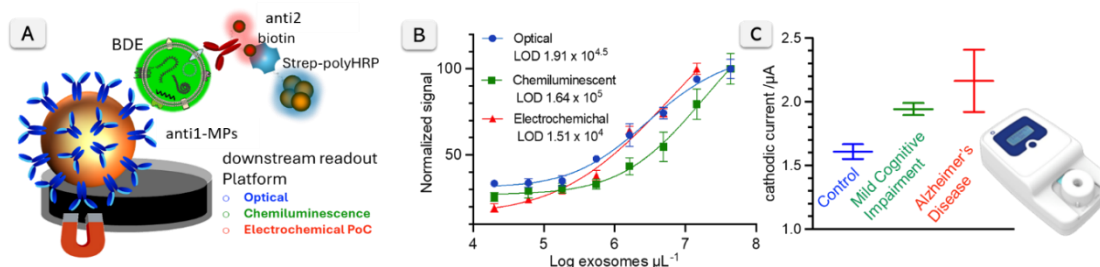
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Alzheimer's Disease (AD) is a leading cause of dementia, accounting for 60-70% of cases globally as reported by the World Health Organization (WHO). Diagnosing AD is particularly challenging because by the time symptoms manifest, significant neuronal loss and brain atrophy have already occurred. Current diagnostic methods like brain imaging and cerebrospinal fluid analysis are costly and invasive, leading to low patient compliance. Exosomes, nano-sized extracellular vesicles secreted by most cell types, have been shown to carry AD-related molecules and are promising candidates for early diagnosis biomarkers. Although the full molecular mechanisms of AD are not completely understood, early pathological changes in the brain might be reflected by the amount and characteristics of brain derived exosomes (BDEs). There is evidence that exosomes can pass the brain blood barrier and be found in several body fluids, including blood, representing a huge advantage to the current diagnostic targets. This study aimed to explore the use of BDEs in plasma as biomarkers for AD. We optimized our method using exosomes from the SH-SY5Y neuroblastoma cell line, obtained through differential ultracentrifugation. We used tosyl-activated magnetic nanoparticles functionalized with a specific antibody to capture BDEs (anti1-MPs). Subsequently, we targeted a biomarker specific for AD on the surface of the captured exosomes using a biotinylated antibody (anti2-biotin). We developed immunoassay platforms with optical, chemiluminescent, and electrochemical readouts, as depicted in **Figure 1 (A)**. We got very promising results with the 3 platforms, especially with the electrochemical one, which had a lower LOD (**Figure 1, B**). A screen-printed electrode integrated with electrochemical reader has been used for the analysis of plasma patients with Mild Cognitive Impairment and suspected Alzheimer's Disease. Preliminary results showed the capability of the method for risk stratification of the patients (**Figure 1, C**).

**Keywords:** Exosomes, in-vitro diagnostic test, biosensors, magnetic particles, Alzheimer's disease.



## Graphene-based Nanomaterials Integration with Bioreceptors for Enhanced Point-of-Care Diagnostics and Environmental Sensors

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We explore the use of graphene-based nanomaterials combined with bioreceptors to develop advanced Point-of-Care and environmental monitoring devices. We produce nanostructured electrodes through an eco-friendly, cost-effective, one-step printing/stamping technique. Utilizing an infrared laser, we achieve precise fabrication of highly exfoliated reduced graphene oxide (rGO) decorated in situ with gold nanoparticles (AuNPs) via instantaneous laser-induced co-reduction of graphene oxide (GO) and gold cations (Au<sup>3+</sup>). These nanocomposites can be transferred onto flexible substrates like PET or nitrocellulose [1]. The produced electrode can be functionalized with DNA probes, aptamers, antibodies [2], or nanobodies through various chemical methods for the specific detection of target nucleic acids, proteins, or small molecules. Binding events between immobilized bioreceptors and target analytes cause detectable changes in the electrochemical signal, enabling rapid and sensitive detection of biomarkers or environmental pollutants. The inclusion of AuNPs on the rGO surface enhances electrochemical performance by increasing the number of active sites, thereby improving sensitivity in biosensor applications, while the biorecognition elements provide high specificity. This integration of highly specific bioreceptors, graphene-based nanomaterials and electrochemical techniques represents a promising strategy for point-of-care applications ranging from environmental monitoring to health diagnostics.

### Acknowledgments:

This project is partly funded from the European Union's Horizon Europe – the Framework Programme for Research and Innovation (2021–2027) under grant agreement No 101120706 (2D-BioPAD), from the HORIZON-WIDERA-2021-ACCESS-02-01 under grant agreement No 101059266 (SUSNANO), and from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101029884 (SERENA). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them. The ICN2 is funded by the CERCA programme/Generalitat de Catalunya, supported by the Severo Ochoa Centres of Excellence programme, Grant CEX2021-001214-S, funded by MCIN/AEI/10.13039.501100011033. We acknowledge Departament de Recerca i Universitats of Generalitat de Catalunya for the grant 2021 SGR 01464 and Grant PID2021-124795NB-I00 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe".

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## Point-of-Care Haemoglobin Detection for Anaemia Diagnosis

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Anaemia is a blood-related disease affecting people of all ages, genders, and ethnicities. It is caused by a deficiency in the number or quality of erythrocytes; or haemoglobin concentration in blood, resulting in a deficit in oxygen transport. Anaemia is often a symptom of other diseases, which can make its diagnosis difficult <sup>[1]</sup>. Anaemia can be classified into different phenotypic groups, such as: haemolytic, microcytic, macrocytic, hypochromic, and Iron Deficiency Anaemia (IDA), among others <sup>[2,3]</sup>. These have different causes and treatments and its diagnosis typically involves the measurement of several key biomarkers in patients' blood, such as haemoglobin (Hb) concentration, erythrocytes' physical parameters, serum iron and serum ferritin levels. Current methods for anaemia diagnosis rely on blood analysis and a complete haemogram. Herein, we are comparing two different nanobiosensors for the electrochemical detection of Hb based: on one hand, the interaction between methylene blue (MB) and Hb and, on the other hand, the complexation of Hb with its aptamer <sup>[4]</sup>. These detection methods could be used for developing a Point-of-Care (PoC) biosensor, which will be user-friendly, fast, and less invasive, requiring only a small drop of blood. Additionally, it could serve as a screening and monitoring tool for other disease states in which anaemia is a symptom.

### Acknowledgements:

ICN2 is funded by CERCA programme, Generalitat de Catalunya. Grant SEV-2017-0706 funded by MCIN/AEI/10.13039/501100011033. This project has received funding from NanoAnaemia, Grant PLEC2021-007727 funded by MCIN/AEI/10.13039/501100011033 and by the "European Union NextGenerationEU/PRTR".

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## Aptamer-Based Inkjet-Printed Nanostructured Biosensors for Real-Time Environmental Monitoring of Antibiotics

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The pervasive presence of antibiotics in the environment poses a significant threat to ecosystems and public health, necessitating real-time monitoring solutions.

Here, we propose an aptamer-based inkjet-printed nanostructured biosensors designed for the sensitive and selective detection of antibiotics in environmental matrices. Indeed, the use of advanced nanostructured materials, electrode fabrication methods, and aptamer-based recognition offers an efficient method for real-time monitoring of antibiotics.

Inkjet-printed electrodes offer a reliable and cost-effective alternative for developing electroanalytical platforms that are scalable, highly reproducible, and versatile in terms of materials. This approach allows precise deposition of nanomaterial-based inks and optimization of biosensor design and performance <sup>[1]</sup>. The performance of a biosensor is closely related to the interactions between its surface elements, and enhancing these interactions is vital for maximizing sensitivity and selectivity <sup>[2]</sup>. An aptamer is a short, single-stranded DNA or RNA molecule that binds specifically to a target molecule. The use of aptamers in electrochemical sensors offers several advantages, such as high specificity, stability, reusability, low cost. Furthermore, aptamers can be easily chemically modified for improved performance in electrochemical detection systems. By labelling the aptamer at one extremity with a thiol group and with methylene blue at the other extremity, the gold working electrode can be easily functionalized with the aptamers through the thiol-gold chemistry and detect the presence of the antibiotic analyte, through the difference in the electron transfer observable in the absence and in the presence of the target. This platform has the potential for in-field applications in order to detect antibiotic contamination in environmental samples and solve ecological and related public health issues.

### Acknowledgments:

This project is partly funded from the HORIZON-WIDERA-2021-ACCESS-02-01 under grant agreement No 101059266 (SUSNANO).

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