References

[1] "Facts and figures at a glance," National Spinal Cord Injury Statistical Center, Tech. Rep., 2020.
[2] A. Villanueva and R. Cabeza, "A novel gaze estimation system with one calibration point," *IEEE Trans. Syst., Man, Cybern. B*, vol. 38, no. 4, pp. 1123–1138, 2008. doi: 10.1109/TSMCB.2008.926606.
[3] E. D. Guestrin and M. Eizenman, "General theory of remote gaze estimation using the pupil center and corneal reflections," *IEEE Trans. Biomed. Eng.*, vol. 53, no. 6, pp. 1124–1133, 2006. doi: 10.1109/TBME.2005.863952.
[4] A. T. Duchowski, *Eye Tracking Methodology: Theory and Practice*. New York: Springer-Verlag, 2007.
[5] L. S. Pedrotti and F. L. Pedrotti, *Optics and Vision*. Englewood Cliffs, NJ: Prentice Hall, 1998.
[6] S.-W. Shih and J. Liu, "A novel approach to 3-D gaze tracking using stereo cameras," *IEEE Trans. Syst., Man, Cybern. B*, vol. 34, no. 1, pp. 234–245, 2004. doi: 10.1109/TSMCB.2003.811128.
[7] Z. Zhu and Q. Ji, "Novel eye gaze tracking techniques under natural head movement," *IEEE Trans. Biomed. Eng.*, vol. 54, no. 12, pp. 2246–2260, 2007.

Nicolas Moser, Jesus Rodriguez-Manzano, and Pantelis Georgiou, *Member, IEEE*

ProtonDx: Accurate, Rapid and Lab-Free Detection of SARS-CoV-2 and Other Respiratory Pathogens

Abstract

ProtonDx will provide a response to the COVID-19 pandemic by bringing nucleic-acid based molecular diagnostics to the palm of your hand. It will support the deployment of the Lacewing technology, which achieves accurate, rapid, handheld and low cost detection of SARS-CoV-2 and other respiratory infections. Results are synchronized to electronic health records and geotagged for real-time surveillance of disease progression. The device was designed for use at the point of need, in places such as pharmacies, schools and workplaces. Its unique approach combines standard semiconductor technology, advanced molecular biology and 3D printed microfluidics to match the performance of a bench-based instrument. Clinical trials are currently in progress at Imperial NHS Trust, London, UK which will lead to regulatory approvals and commercialization in the next few months.

I. Introduction

The emergence of SARS-CoV-2 in the late months of 2019 has triggered a new pandemic which has infected over 50 million people and caused over 1.5 million deaths at the time of writing this article, significantly straining health systems across the world. It has also caused drastic changes to our daily lives with country-wide lockdowns and travel restrictions across 2020, severely damaging the economy. Since the start of the pandemic, rapid testing has always been at the forefront of a path to recovery, while the research for a vaccine was ongoing. Governments have often set up a response strategy based on large-scale testing and contact tracing, with the intention to prevent the spread of the infection. These tests have been associated with numerous issues, among which inaccuracies and inaccessibility have appeared as the main challenges. In the UK, it is estimated that home tests require 4 days to receive results and patients need to travel just under 10 km on average to reach a testing site. New diagnostic technology is essential to provide efficient testing as a response to the COVID-19 pandemic.

There are mainly two types of tests available for COVID-19, viral and antibody tests. The viral tests are direct tests which are designed to detect the virus by targeting SARS-CoV-2 viral RNA or antigens, and therefore indicate current infection. On the other hand, antibody tests are indirect tests that measure established seroconversion to previous infection, or early seroconversion to ongoing infection. Figure 1 emphasizes the time relationship between diagnostic test and evolution of COVID-19 infection.

Great efforts have been made in order to develop rapid antigen test for SARS-CoV-2. However, the principal concerns are the false-negative rate due to inadequate limit of detection. The recommended test for diagnosis of SARS-CoV-2 infection involves detection of viral RNA using nucleic acid amplification tests, such as reverse transcription (RT)-PCR.

Our cutting-edge research at the interface of electronics, molecular biology and microfluidics at Imperial College London has enabled the development of

Digital Object Identifier 10.1109/MCAS.2021.3092586
Date of current version: 12 August 2021

Lacewing, a nucleic acid amplification test which offers the best of both worlds: high accuracy, high speed and low cost, without a lab and in the palm of your hand. Lacewing is a handheld test which connects to a smartphone to provide results from a swab in under 20 minutes and synchronize the results with electronic health records (see Fig. 2). This article describes our state-of-the-art approach to diagnostics and our entrepreneurial journey towards developing ProtonDx as a spinout.

II. Lacewing: A Lab-Free Molecular Test

Lacewing's unique capabilities can be summarized as accurate, rapid, handheld, low cost and multi-infection. It aims to bring the capabilities of a molecular lab to the palm of your hands. Lacewing allows for rapid testing of COVID-19 and other respiratory infections where it is needed: in pharmacies, at work, at school, in care homes, and eventually in your own home. Fig 3 represents the diagnostic workflow. Lacewing relies on a swab, extracting the sample in under 5 minutes to collect the RNA of the virus. When loaded into the device, a molecular reaction called Reverse-Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP) is triggered to reverse-converse and amplify the SARS-CoV-2 RNA. The reaction is facilitated by reagents such as enzymes and primers, and an isothermal heating at 63 °C. Detection is performed using Ion-Sensitive Field-Effect Transistors (ISFETs) in an unmodified CMOS process, leveraging the economies of scale of the semiconductor industry into the world of diagnostics. The microchip integrates an array of 78 × 56 ISFETs which monitor the release of protons during DNA amplification and dedicated data processing algorithms are applied to identify the signal associated with the amplification. The results are sent in under 15 minutes to our smartphone app, detecting the presence of SARS-CoV-2 in the patient sample. The data is then synchronized to electronic health records, and geo-tagged on a map for real-time surveillance of the pandemic. The map is available online as a web interface, reporting automatically and anonymously all tests run by Lacewing anywhere in the world. This is essential for health organizations and governments, providing essential data to help plan a strategy for management of the pandemic.

III. The Story of Lacewing

Lacewing is a case of application of circuits and systems into healthcare to address a global health challenge. It has indeed been validated with a wide range of molecular targets, including antimicrobial resistance [1], aspergillus [2] and tropical diseases [3]. But more than a test for diagnosis diseases, Lacewing is a state-of-the-art electrochemical imaging platform

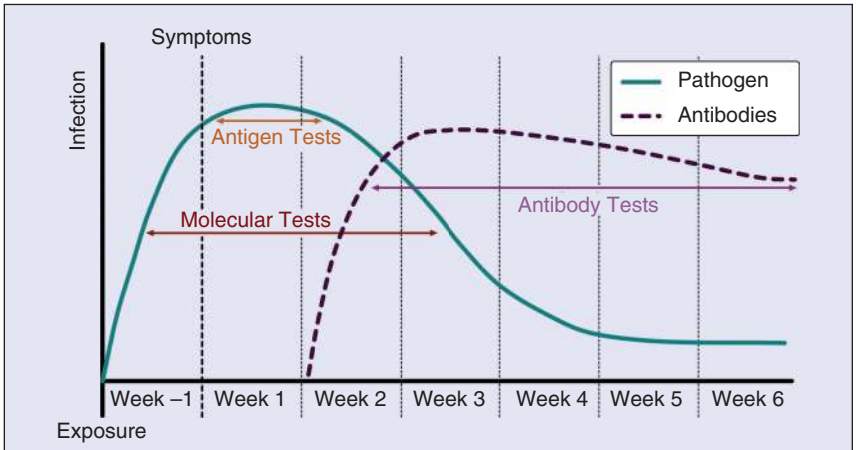

Figure 1. Evolution of the COVID-19 infection during the weeks following exposure.

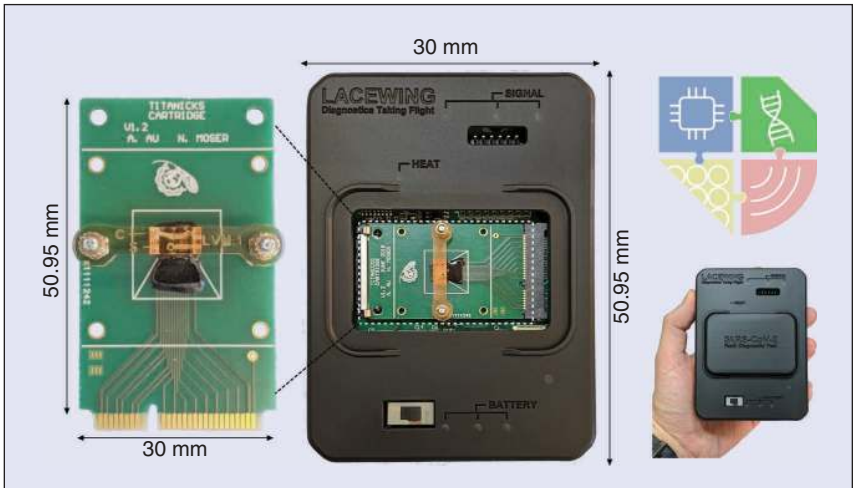

Figure 2. The time relationship between diagnostic tests and COVID-19 infection.

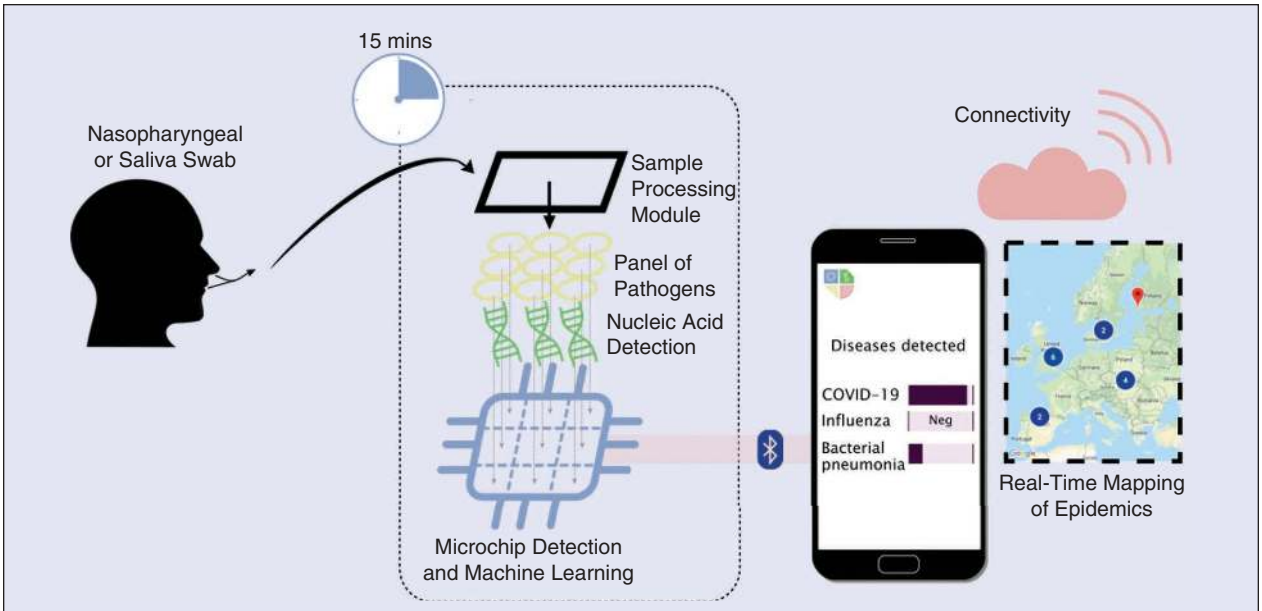

Figure 3. Diagnostic workflow from sample to result.

developed during years of research into Ion-Sensitive Field-Effect Transistors (ISFETs) in unmodified CMOS technology, in Dr Georgiou's group at the Centre for Bio-Inspired Technology, Imperial College London.

A. ISFET-Based CMOS Microchip

Over the past decades, ISFETs have appeared as ideal candidates for potentiometric sensing due to monolithic integration with CMOS instrumentation [5]. When structured in arrays, ISFETs allow for electrochemical imaging, acting as a real-time camera into chemical reactions. ISFETs are well-known for suffering from several non-idealities particularly when implemented in unmodified CMOS technology, including trapped charge and drift. In the group, we have focused on implementing advanced compensation schemes at the sensor level using dedicated analogue front-end. In 2018, we designed TITANICKS (Fig. 5), a new microchip where the pixel architecture encodes the chemical signal in the time domain for high resolution and integrates a RAM for sensor compensation [4]. With this chip we also demonstrated our first LAMP assay detection.

B. Handheld Device for Infectious Disease Diagnostics

We designed a Lab-on-Chip (LoC) cartridge to interface electronic and molecular biology (Fig. 5). The cartridge relies on laser cut acrylic to form a reaction chamber, with a thin chloridized silver wire acting as Ag/AgCl reference electrode. The cartridge interfaces to the main board for data acquisition using a microcontroller and communication to a phone via Bluetooth. Thermal control is also enabled using a Peltier module, on-chip temperature sensors and a PID controller [4].

Because our test is handheld and battery-powered, it is ideally suited to application in the community or low-resource settings, hence our vision for Lacewing was targeted at tropical diseases in low- and middle-income countries. We

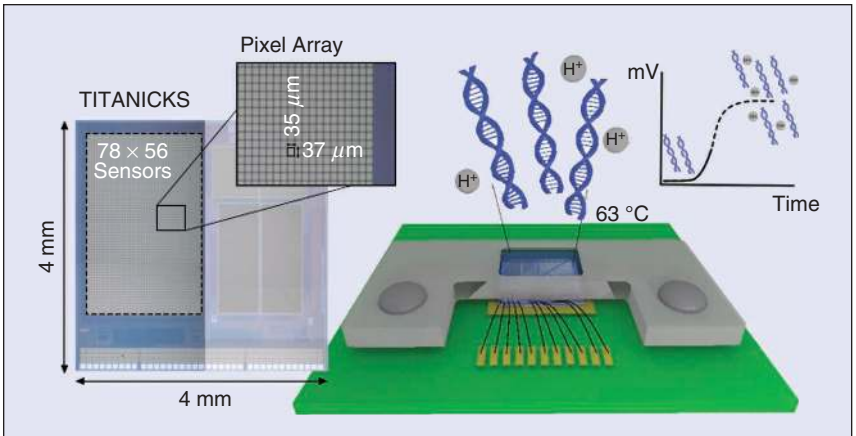

Figure 4. CMOS microchip integrating over 4,000 ISFET sensors integrated within a Lab-on-Chip package for nucleic acid amplification and detection [4].

embarked on a first validation for dengue diagnosis at Kaohsiung Medical Hospital, Taiwan, in November 2018. In July 2019, we headed to the University of Ghana and validated Lacewing with malaria clinical samples, achieving detection in under 20 minutes. In parallel, we demonstrated Lacewing for detection of aspergillus in environment samples in London [2]. While these trials gave us confidence that our platform could detect a wide range of pathogens, we went through an exercise of rapid adaptation at the end of 2019. We developed a rapid response to the emergence of a new strain of drug-resistant bacteria carrying the mobilized colistin resistance (*mcr*-9) gene, adapting the test and publishing the results of the clinical validation in under 3 months [6]. So when COVID-19 emerged, we were ready.

C. The COVID-19 Pandemic

The team spent the lockdown in the lab repurposing Lacewing for detection of SARS-CoV-2. We took this opportunity to develop new microfluidic capabilities using 3D printing to achieve two reactions in parallel on each cartridge, a sample and a control (see Fig 2).

During the study, we ran with 183 clinical samples from Charing Cross hospital and showed performance of over 90% sensitivity (true positive) and 100% specificity (true negative). We achieved quantitative detection on Lacewing in under 13 minutes on average [1]. Since the lockdown, we have improved our LAMP assay and have now achieved 97% sensitivity and detection in under 10 minutes, with positives as early as 5 minutes. We have also developed a frugal sample processing method to interface with Lacewing and extract viral RNA in under 5 minutes. Our sample-to-result workflow is now achievable in up to 15 minutes. We are currently working towards multiplexing 5–10 respiratory infections, which will be achieved by combining advances in microfluidic design to integrate more wells on the chip, and data-driven methods by leveraging kinetics of the reaction [?]. The performance is summarized in Fig. 6.

IV. Market

The market of point-of-care (PoC) diagnostics has been gaining momentum with a current valuation of $9 billion worldwide, shifting from the wider market of in vitro diagnostics (IVD) was worth 41 billion worldwide in 2017 (*Source: EvaluateMedTech*). This does not consider the consequences of the COVID-19 pandemic, which will significantly accelerate market growth due to urgent need for rapid PoC diagnostics. In the UK alone, the demand for COVID-19 test in September 2020 was up to 3 times the current capacity i.e. about 250,000 hospital tests (unit cost approximately $13) and 650,000 home tests (unit cost approximately $20) per day. While it is hard to predict the demand for COVID-19 tests across 2021, particularly with the commercialization of the vaccine, a stable demand would lead to a market worth of $6.15b a year, which could range anywhere from $3b to $50b depending on the status of the pandemic in the upcoming year.

Since the emergence of COVID-19, many large players in diagnostics including Abbott, Roche and Biomerieux have released molecular assays and platforms for diagnostics, but they all require lab access which incurs delays. These fall into the category of PCR instruments in Table Fig. 7. In contrast, portable diagnostics have been dominated by antigen tests which are simple, low cost and scalable but suffer from a limit of detection of

Figure 5. Validation for malaria diagnosis at the University of Ghana, Accra, Ghana. PhD students Kenny Malpartida-Cardenas and Ivana Pennisi are shown loading samples into several Lacewing devices.

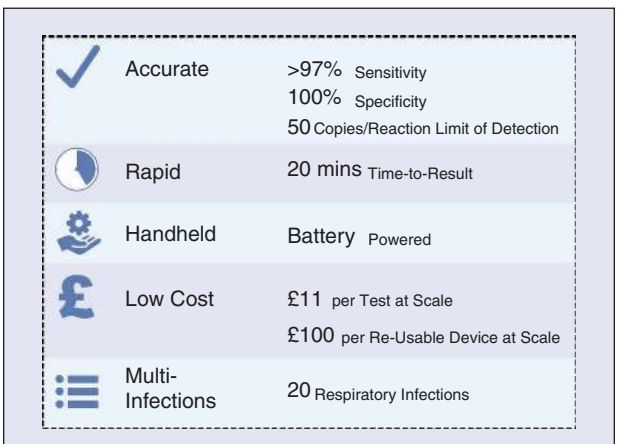

Figure 6. Specification of Lacewing as a COVID-19 test.

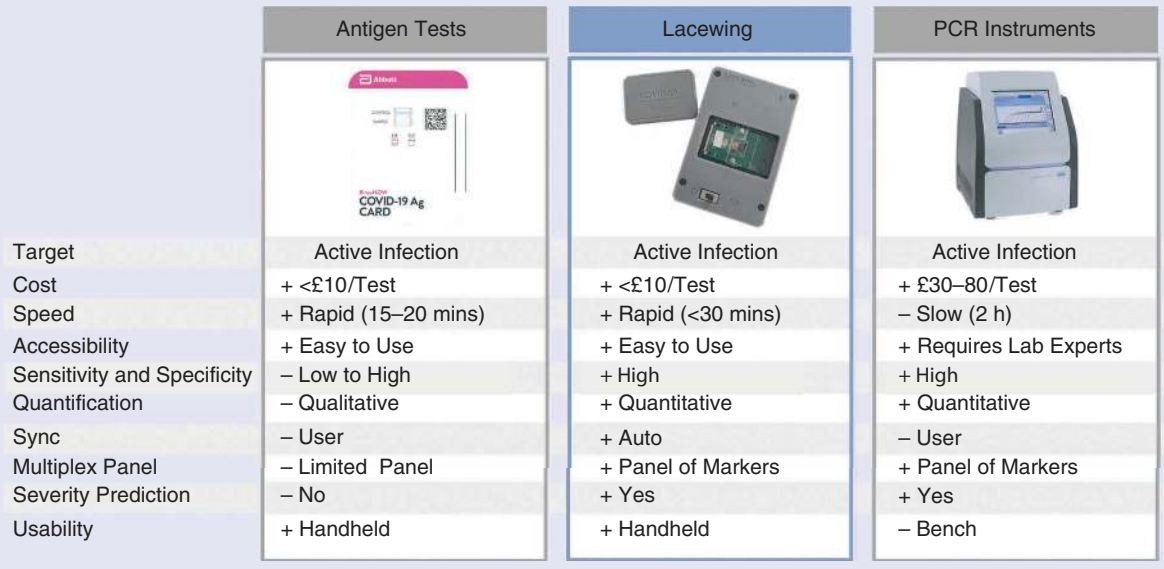

Figure 7. Performance of Lacewing against state-of-the-art antigen and nucleic acid based tests.

several orders of magnitude higher than molecular tests i.e. they are prone to false negatives in the early days of the infection. Companies such as LumiraDx have developed a benchtop device to provide results without the need for visual interpretation, at the expense of portability. Lacewing is the first handheld molecular platform able to perform the test in under 15 minutes without the need for a lab.

V. ProtonDx

Along with developing and validating Lacewing as a COVID-19 molecular test, we have initiated the process of spinning out from Imperial College London as ProtonDx Ltd, to support scale-up of testing capabilities and commercialization of the platform. We are currently running clinical trials at Imperial NHS Trust in London, UK, during which we are testing the platform with 400 swab samples. We are setting up a supply chain to scale up the production of our handheld device and test cartridges.

Our first stage of deployment will see us implement Lacewing within emergency services and maternity wards in hospitals. We will deploy in pharmacies and health services to provide a touchpoint for testing in the community as an alternative to home testing. Lastly, we will provide testing in schools and workplaces, where batch testing can be used to guarantee an infection-free zone and contain the virus.

In a close future, ProtonDx will bring diagnostics of COVID-19 to the palm of your hand, finally allowing to diagnose patients at the time and point where those first symptoms occur.

References

[1] J. Rodriguez-Manzano, P. Y. Chia, T. W. Yeo, A. Holmes, P. Georgiou, and S. Yacoub, "Improving dengue diagnostics and management through innovative technology," *Curr. Infect. Dis. Rep.*, vol. 20, no. 8, p. 25, June 2018. doi: 10.1007/s11908-018-0633-x.

[2] L. S. Yu et al., "Rapid detection of azole-resistant aspergillus fumigatus in clinical and environmental isolates by use of a lab-on-a-chip diagnostic system," *J. Clin. Microbiol.*, vol. 58, no. 11, Nov. 2020. doi: 10.1128/JCM.00843-20.

[3] K. Malpartida-Cardenas et al., "Quantitative and rapid Plasmodium falciparum malaria diagnosis and artemisinin-resistance detection using a CMOS Lab-on-Chip platform," *Biosens. Bioelectron.*, vol. 145, Dec. 2019. doi: 10.1016/j.bios.2019.111678.

[4] N. Moser, J. Rodriguez-Manzano, T. S. Lande, and P. Georgiou, "A scalable ISFET sensing and memory array with sensor auto-calibration for on-chip real-time DNA detection," *IEEE Trans. Biomed. Circuits Syst.*, vol. 12, no. 2, pp. 390–401, 2018. [Online]. Available: https://ieeexplore.ieee.org/abstract/document/8278828/. doi: 10.1109/TBCAS.2017.2789161.

[5] N. Moser, T. S. Lande, C. Toumazou, and P. Georgiou, "ISFETs in CMOS and emergent trends in instrumentation: A review," *IEEE Sens. J.*, vol. 16, no. 17, pp. 6496–6514, Sept. 2016. [Online]. Available: https://ieeexplore.ieee.org/document/7501581/. doi: 10.1109/JSEN.2016.2585920.

[6] J. Rodriguez-Manzano et al., "Rapid detection of mobilized colistin resistance using a nucleic acid based lab-on-a-chip diagnostic system," *Sci. Rep.*, vol. 10, no. 1, Dec. 2020. doi: 10.1038/s41598-020-64612-1.