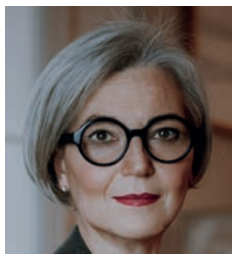
Single-molecule detections at point-of-care: is this a dream?



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A healthcare system that addresses the healthy ones, is a visionary concept aiming to establish a proactive healthcare system that benefits all living beings on Earth. The core idea involves early identification of asymptomatic individuals upon the onset of illness, enabling clinicians to intervene swiftly and effectively. This proactive approach contrasts with the current reactive model, where treatment primarily begins after symptoms appear, leading to a more efficient and cost-effective system.

The key to this approach lies in identifying specific biomolecules, such as proteins, nucleic acids which serve as markers. These are measurable indicators that signal the presence or severity of a disease. They can be detected in various bodily fluids like saliva, mucus, serum, plasma, and urine, providing early diagnostic capabilities.

Among biomarkers, molecular biomarkers, including oligonucleotide-based strands (DNA, RNA, mRNA) and proteins/antigens, are commonly utilised. These molecules selectively bind to specific probes or antibodies, enabling their detection with high sensitivity.

Biomarkers serve multiple purposes, including identifying asymptomatic individuals, characterising the different stages of disease progression with precision, and serving as targets in clinical trials. They are crucial not only in detecting tumoral conditions but also in diagnosing neurological pathologies, viral, or bacterial infections.

By leveraging biomarkers and advanced detection methods this approach to healthcare seeks to revolutionise its management, promising to enhance population well-being. Embracing this proactive paradigm shift holds the potential to transform our current reactive sick-care system into a proactive healthcare system, benefiting all living species on our planet.

Cancer diagnosis traditionally relies on invasive tissue biopsies, yet liquid biopsies offer significant advancements by detecting markers in blood sample in a minimally invasive, cost-effective, and allow continuous monitoring of tumour evolution and treatment response. However, biomarker concentrations in peripheral fluids are much lower than in tumour masses, posing a challenge.

The possibility to combine ultra-portable, handheld, technologies for point-of-care testing (POCT), emphasising single-molecule detection capabilities with diagnostic sensitivity and selectivity above 95%, would be a dream come through and an extremely useful tool for reliable screening of asymptomatic individuals. Technologies like Single-Molecule-with-a-large-Transistor (SiMoT) (E. Genco, at al. Adv. Mater. 2023, 35, 2304102) and Clustered-Regularly-Interspaced-Short-Palindromic-Repeats (CRISPR) (R. Zhu et al. Anal. Chim. Acta 2023, 1257, 341175) offer multiplexing capabilities, detecting various diseases by altering recognition elements. CRISPR targets oligonucleotide biomarkers, while SiMoT detects both proteins and oligonucleotides. These technologies utilise fast, handheld devices with disposable cartridges tailored to specific applications. Additionally, they hold promise for use in low-resource settings and during health crises like epidemics.

The vision of a comprehensive healthcare system that extends its focus beyond human health to encompass animals and plants is depicted in *Figure 1*. In this envisioned scenario, individuals, whether they are patients or farmers, can collect samples such as saliva, blood, urine, or sap from animals or plants in a remote location. These samples are then placed on a disposable cartridge, such as the bioelectronic SiMoT cartridge, which is connected to a handheld electronic reader resembling a glucometer. This reader can transmit data to a smart device via Bluetooth or USB, which in turn uploads the data to a cloud platform. An artificial intelligence algorithm analyses the data, and the results are relayed back to the patient's smart device. This process can be adapted for various POCT technologies, including lateral flow devices based on the CRISPR principle.

To realise this dream healthcare ecosystem, comprehensive screening of the general population, including all living organisms, using POCT technologies is crucial. Early screening is a cornerstone of preventive medicine, enhancing treatment success rates for diseases like cancer, cardiovascular conditions, and diabetes. Early detection allows for more effective and less invasive treatment options, leading to improved patient outcomes and reduced disease progression. Moreover, early screening can lower healthcare costs by avoiding expensive interventions and hospitalisations associated with advanced disease stages. It also improves quality of life by preventing complications and reducing the impact of symptoms on daily activities.

Screening is also vital for preventing the spread of infectious diseases. Identifying and isolating infected individuals early can contain outbreaks and protect public health. Additionally, screening contributes to health equity by ensuring timely and effective healthcare for individuals from all backgrounds.

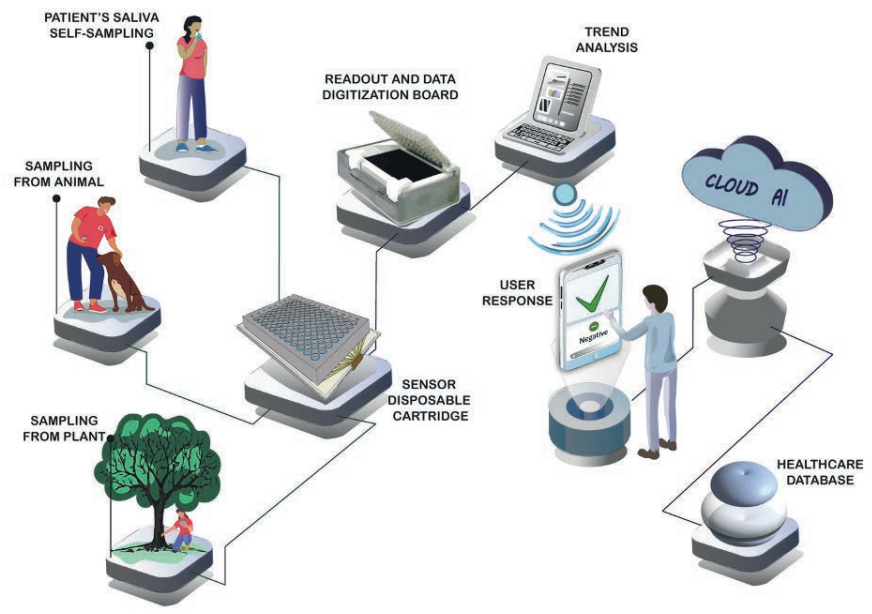

Figure 1: The vision of the asymptomatic screening with a POCT technology in a one-healthcare ecosystem (reproduced with permission from ref. E. Macchia et al., Adv. Mater. 2024, 36, 2309705.

POCT technologies play a crucial role in implementing widespread screening programs. The World Health Organization has established criteria for ideal POCT technologies, summarised by the acronym 'REASSURED'. (K. J. Land, at al. Nat. Microbiol. 2019, 4, 46). These criteria include affordability, sensitivity, specificity, user-friendliness, rapid results, robustness, equipment-free operation, connectivity, real-time insights, minimal sample requirements, versatility, stability, embedded quality control, regulatory approval, scalability, diagnostic range, and global accessibility.

Integrating POCT screening with the one-health approach acknowledges the interconnectedness of human, animal, and environmental health. This integration can guide screening efforts in populations exposed to zoonotic diseases and aid in epidemiological surveillance across species. Monitoring wildlife populations is also relevant for detecting and preventing the spread of diseases.

Realising the vision of a comprehensive healthcare system that considers the health of all living organisms requires widespread screening using POCT technologies. These technologies must meet stringent criteria to ensure effectiveness, accessibility, and ethical use. Integrating screening efforts with the one-health approach can enhance disease prevention, surveillance, and control, ultimately promoting health and well-being for all living beings and the ecosystems they inhabit.

The SiMoT technology represents a significant leap forward in point-of-care testing (POCT), with two platforms reaching Technology Readiness Levels (TRL) 5-6, indicating validation in relevant environments and pre-clinical studies. These platforms offer distinct advantages, catering to different needs in healthcare settings. One platform features a single biofunctionalized electrode, ideal for assessing single markers/pathogens, while the other is a 96-well ELISA-like array capable of multiplexing, enabling simultaneous analysis of multiple markers, such as those found in the blood of pancreatic cancer patients.

The single-sensor platform is particularly noteworthy for its user-friendly design and cost-effectiveness. It is engineered to be accessible even to untrained individuals, making it suitable for use in remote or resource-limited settings commonly found in underdeveloped countries. On the other hand, the array technology, while more

complex, offers enhanced capabilities and is better suited for use by trained personnel in clinical or decentralised settings.

Both platforms operate on the principle of capacitive coupling between a functionalised gate and a polarising conducting system. This interaction induces a shift in threshold voltage (VT) and transistor current (ID) upon binding of markers to the biofunctionalized gate. The disposable accessory cartridges, integral to both platforms, incorporate biofunctionalized gates specific to the marker being detected, as well as non-biofunctionalized reference gates to ensure stability during the assay. These cartridges are customised for different applications, ranging from detecting pathogens like the SARS-Cov2 virus in self-sampled saliva to diagnosing pancreatic cancer precursors in blood plasma.

Connectivity is a key feature of SiMoT technology. Electronic readers are seamlessly integrated with smart devices via Bluetooth or USB, enabling direct data transfer and facilitating real-time analysis. This connectivity streamlines data management, analysis, and reporting, enhancing the overall efficiency of the testing process. Moreover, SiMoT is designed to be ultra-portable and handheld, operating in fully equipment-free environments with minimal infrastructure requirements.

Sustainability is a core consideration in the development of SiMoT technology. Efforts are underway to explore the use of biodegradable materials derived from biomass and food waste, along with additive printing techniques, to minimise environmental impact. This sustainable approach extends to the design of fully recyclable, battery-less Si-integrated circuit readers, further reducing the ecological footprint of the technology.

Scalability is another crucial aspect of SiMoT technology. The platforms have demonstrated exceptional performance, achieving single-molecule detection limits with diagnostic sensitivity and selectivity exceeding 95%. This level of performance has significant implications for disease diagnosis, particularly in less developed countries where access to healthcare resources is limited. SiMoT's rapid assay capabilities, delivering results in approximately 20 minutes for single markers and an hour for the array, further enhance its utility in clinical settings.

SiMoT's versatility extends to its ability to assay both proteins/antigens and nucleic acid strand markers simultaneously, offering a comprehensive approach to disease diagnosis. The technology's unique combination of features, including the amplification effect, Boolean assay operation, and utilisation of Artificial Intelligence algorithms, sets it apart as a superior POCT technology, surpassing existing technologies like CRISPR-based assays.

In terms of cost-effectiveness, SiMoT offers competitive pricing, with cost estimates indicating affordability for both readers and cartridges. Even in low-resource settings, where the cost of the reader can be shared among multiple patients, SiMoT remains an accessible option for healthcare providers. Moreover, as production scales up, costs are expected to decrease further, making SiMoT even more accessible to a wider population.

Overall, SiMoT technology represents a paradigm shift in POCT, offering unmatched performance, versatility, and sustainability. Its potential to revolutionise disease diagnosis and management, particularly in resource-limited settings, underscores its importance in advancing global healthcare initiatives. With ongoing research and development efforts focused on refining and expanding its capabilities, SiMoT is poised to make a lasting impact on healthcare delivery worldwide.