

A rapid and inexpensive paper-based test for multiplexed sensing of biomarkers

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Deep Learning-Enabled Multiplexed Point-of-Care Sensor using a Paper-Based Fluorescence Vertical Flow Assay. Credit: Ozcan Lab @ UCLA

Centralized laboratory testing has been a standard tool for diagnosing common illnesses. However, laboratory-based testing generally requires expensive medical equipment and involves complex operation protocols that can only be completed within a medical facility by well-trained personnel. These requirements elongate testing time and prevent the widespread use of diagnostics in remote and resource-limited regions due to limited access to centralized labs. To address some of these limitations, point-of-care (POC) sensors were developed as alternative diagnostics tools with simple and rapid operation, compact size, and low cost.

Paper-based [sensors](#) form the most common type of POC tests, often called lateral flow assays (LFAs), where the injected sample fluid flows horizontally and reacts with the specific test regions ("test lines"), generating, for example, a color change. Despite their advantages of simplicity and [cost-effectiveness](#), existing LFAs have several limitations, including lower sensitivity and difficulty in multiplexed testing of disease biomarkers.

To overcome these limitations, researchers at UCLA developed a new paper-based biosensor using a fluorescent multiplexed vertical flow assay to simultaneously and rapidly quantify three cardiac biomarkers from human serum samples. The results of this new multiplexed POC sensor were published in the journal *Small*.

Aydogan Ozcan, Chancellor's Professor of Electrical & Computer Engineering and the associate director of the California NanoSystems Institute at UCLA, led the research in collaboration with Professor Dino Di Carlo of the UCLA Bioengineering Department, Professor Omai Garner, the director of UCLA Clinical Microbiology Lab, Dr. Hyou-Arm Joung, a senior research associate at UCLA and Artem Goncharov, a graduate student at UCLA Electrical & Computer Engineering Department.

The vertical flow design of this new paper-based POC sensor enables to have multiple test regions with up to 100 individual test spots within a single disposable cartridge. "This design essentially allows us to integrate tens of different POC sensors into a single cassette and perform multiplexed diagnostics tests in parallel with the same low-cost paper-based sensor," said Prof. Ozcan.

This powerful POC sensor operates using a small droplet of serum and has simple operation steps that a minimally trained user can perform in less than 15 min of total assay time per patient. In addition to

multiplexing, this paper-based sensor also presents high sensitivity, achieving a detection limit better than ~ 0.5 ng/mL for each biomarker—smaller than one billionth of half a gram per milliliter of serum.

In addition to the paper-based vertical flow assay, UCLA researchers also developed a mobile phone-based inexpensive hand-held fluorescence reader and deep learning-assisted signal analysis pipeline to automatically perform accurate quantification of the three target biomarkers in a user-friendly manner.

"Compared to a commonly used linear calibration method, our deep learning-based analysis benefits from the function approximation power of neural networks to learn non-trivial relationships between the multiplexed fluorescence signals from the paper-based sensor and the underlying analyte concentrations in serum," said Goncharov. "As a result, we have accurate quantitative measurements for all three biomarkers of interest despite the background noise present in clinical serum samples."

The researchers tested their paper-based multiplexed sensor for the quantification of three biomarkers of acute coronary syndrome (ACS), including myoglobin, creatine kinase-MB (CK-MB), and heart-type fatty acid binding protein (FABP). ACS is a [cardiovascular disease](#) that requires early diagnosis under an emergency, and these target markers are released into the bloodstream early on after the onset of the symptoms.

The developed paper-based sensor was tested on human serum samples, and the measured concentrations for all three cardiac biomarkers showed a good match with the ground truth measurements obtained by a standard benchtop laboratory test. With its accuracy, rapid operation, ease-of-use, and low cost, this deep learning-enabled paper-based

multiplexed sensor forms an appealing POC test for various applications in remote and low-resource settings.

More information: Artem Goncharov et al, Deep Learning-Enabled Multiplexed Point-of-Care Sensor using a Paper-Based Fluorescence Vertical Flow Assay, *Small* (2023). [DOI: 10.1002/sml.202300617](https://doi.org/10.1002/sml.202300617)

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