Field-based patient trial for cell-free Zika testing delivers highly accurate results

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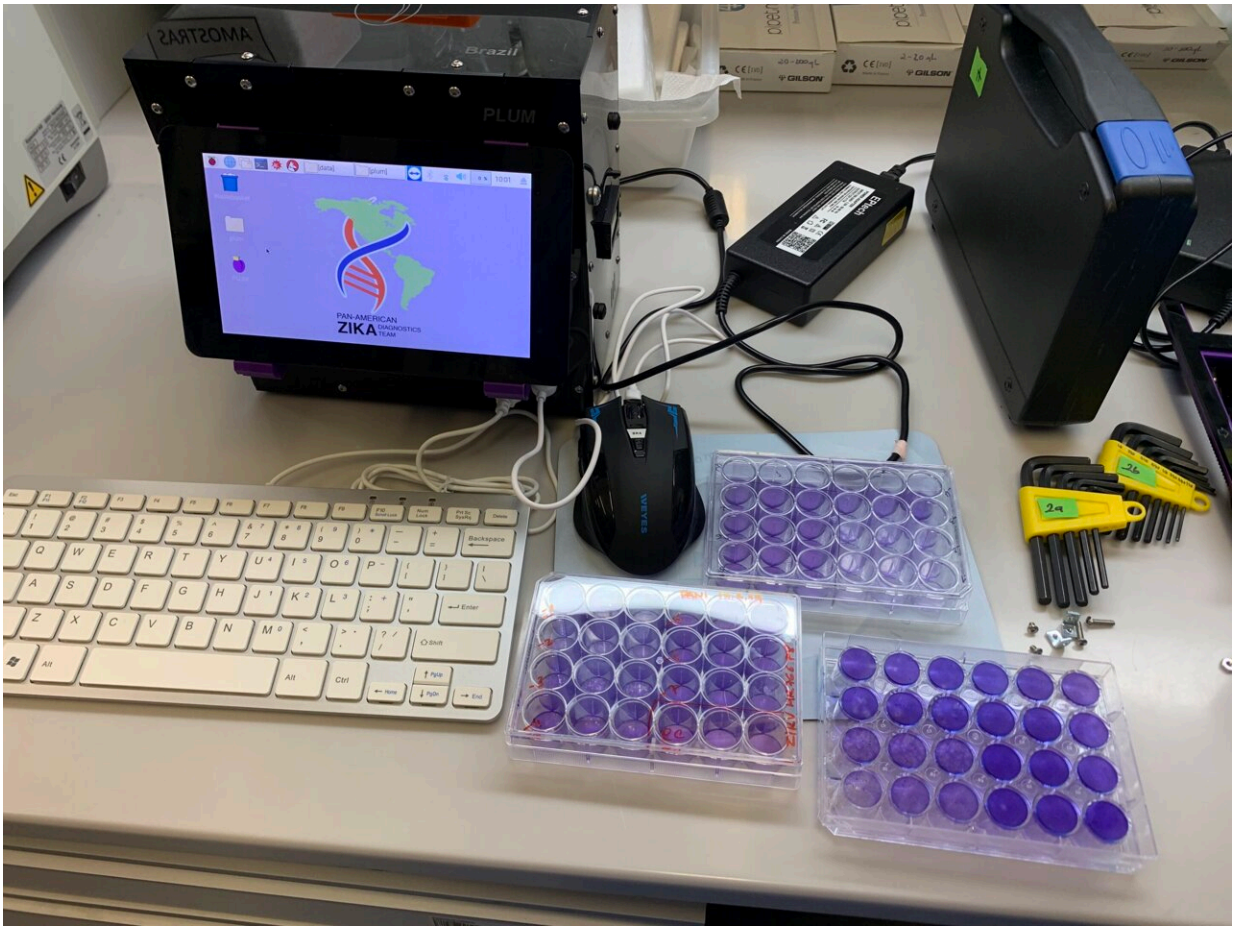

A "lab-in-a-box", the PLUM reader (Portable, Low-cost, User-friendly, Multimode), presents results from up to 384 patient samples and displays them in a single image capture. PLUM is also highly programmable and can be similarly applied to detect any pathogen sequence. Credit: Livia Guo, LSK Technologies

Study results, published today in *Nature Biomedical Engineering*, show that the novel diagnostic platform has analytical specificity and sensitivity equivalent to a US Centre for Disease Control PCR test for Zika and a diagnostic accuracy of 98.5 per cent with 268 patient samples collected in Recife, Brazil. The platform is also programmable and can be similarly applied to detect any pathogen sequence. In addition to validating highly accurate diagnostic results for Zika, the team also achieved similar diagnostic performance for chikungunya virus, another mosquito-borne arbovirus.

"We see emerging diagnostics, like the paper-based tests we've developed, as having tremendous near-term potential to augment existing PCR capacity, improve equity in access to health care, and aid in the responses to public health crises," said Keith Pardee, assistant professor in the department of pharmaceutical sciences, Leslie Dan Faculty of Pharmacy, University of Toronto.

Prior to the current global COVID-19 pandemic, the 2015/2016 outbreak of Zika virus in Latin America emphasized the urgent need for rapid and low-cost testing that can be deployed beyond the reach of centralized diagnostic labs, explains Pardee who has a Canada Research Chair in Synthetic Biology and Human Health. "We were investigating and developing this technology well before the COVID-19 pandemic brought these issues to light at the global level. We've now been able to apply it and validate it in a region of endemic disease, which is really promising because these tools are meant to enable health systems to better respond to future outbreaks of infectious disease, particularly in low-resource settings," he said.

The portable diagnostic platform is a combination of a cell-free, paper-based test and a field-ready companion device that allows data to be collected using image-based color analysis—purple for positive and yellow for negative. Called "PLUM" (Portable, Low-cost, User-friendly,

Multimode), the toaster-size reader presents results from up to 384 samples and displays them in a single image capture. The hardware and software that make up PLUM were originally developed by co-authors Livia Guo and Seray Çiçek as part of their graduate work in the Pardee lab. To keep production costs low, Guo and Çiçek, co-founders of LSK Technologies, used customizable software programs and off-the shelf electronics, enabling PLUM to be built for approximately $500 USD per unit.

On the molecular side, the cell-free tests can be freeze-dried, allowing for distribution without refrigeration and, significantly, all of the molecular components of the test are independent of the PCR-supply chain. "Here we have demonstrated that these two technologies combined create a low-cost, highly accurate diagnostic tool," said study lead author Margot Karlikow, a postdoctoral fellow in the Pardee lab from 2016 to 2021 and now co-founder of En Carta Diagnostics. "We also demonstrated that it is feasible to transport the platform across a significant distance and implement it effectively in another country. In many low- and middle-income countries, there is no PCR testing available outside of main cities, so the ultimate goal is that this platform be used as a high-quality alternative to PCR in more regional settings," she said.

Dr. Lindomar Pena, department of virology, Oswaldo Cruz Foundation (Fiocruz), led the Brazilian team that collaborated on the project. "This robust diagnostic platform displayed desirable features to be used in developing countries such as Brazil and in laboratories with basic infrastructure. We hope it can be further developed and deployed in the Brazilian network of public health laboratories to diagnose Zika patients, trace contacts and identify hot-spot areas with active community transmission," he said.

Showing that the platform could be transported and accurately detect

Zika virus in patient samples is a significant step forward in creating more accessible and de-centralized testing, says Pardee. However, the extraction of RNA from patient samples still requires liquid handling by skilled technicians at this stage. "With performance on patient samples now validated, we are tackling these next challenges, like sample preparation, so that the platform and PCR-like diagnostic capacity can be distributed more broadly into the communities where they are needed."

More information: Keith Pardee, Field validation of the performance of paper-based tests for the detection of the Zika and chikungunya viruses in serum samples, *Nature Biomedical Engineering* (2022). DOI: 10.1038/s41551-022-00850-0

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