CSIR TECHNOLOGY SHOWCASE

Paper-based Diagnostics for COVID-19 Developed by CSIR-IGIB

G. Mahesh

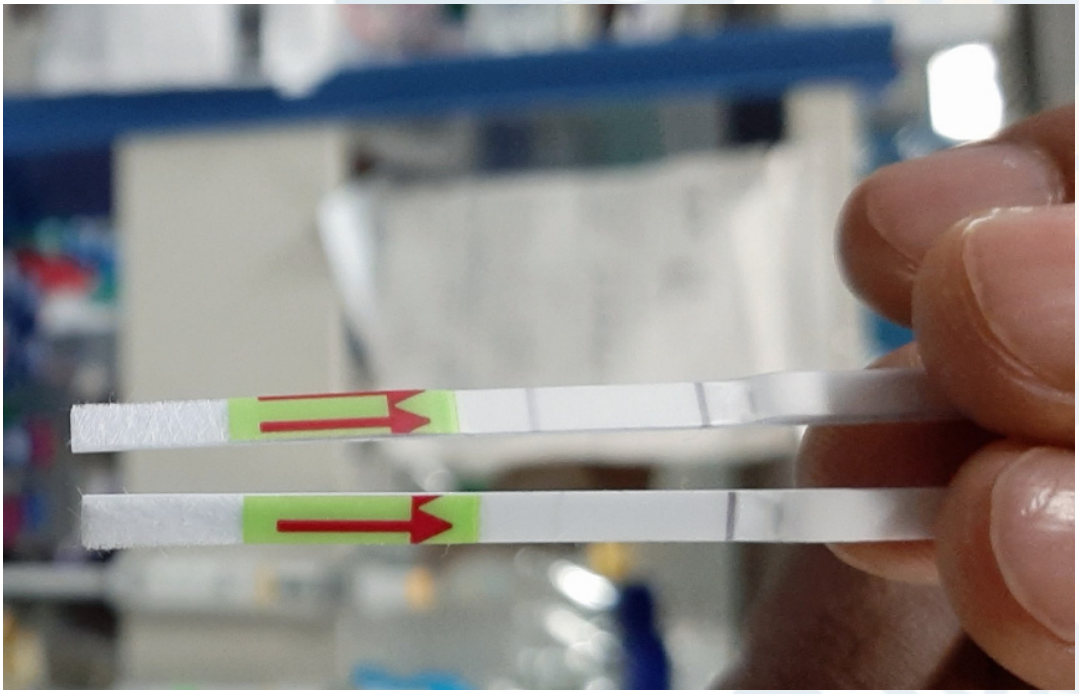

Image credit: Debojyoti Chakraborty, CSIR-IGIB

ON the 3rd of April, the Council of Scientific and Industrial Research achieved a breakthrough in its ongoing war against the rapidly spreading and devastating coronavirus. CSIR's Institute of Genomics and Integrative Biology (CSIR-IGIB) developed a low-cost diagnostic paper-based kit that can accurately test COVID-19 samples.

This timely development has come at a time when COVID-19 diagnostic kits available thus far have been in the price range of Rs 3,500 to Rs 5,000 and had to be primarily imported. In sharp contrast, the CSIR-IGIB breakthrough diagnostic kit is in the order of just Rs 500 per kit with the possibility of the price being further reduced to around Rs 100/-.

Paper-based test kits are not really new. In high school, most of us have undoubtedly used litmus paper strips. The litmus paper strip magically changes colour when dipped into an alkaline or acidic solution. We thus learned it is not magic but pH and also went on to learn about the hows and whys, higher and lower pH, about acids, bases and neutral solutions.

The other well-known paper-based diagnostic kit that is used mostly in the privacy of one's home is the commonly available pregnancy test kit. There are other paper-based kits too that have been developed for detecting

> The paper-based diagnostic kits are lightweight and compact, making it easy for bulk transportability, have low manufacturing costs, and importantly, allow risk-free disposal by incineration.

infectious and non-infectious diseases. It includes some types of cancer, Ebola, Zika, SARS, measles, influenza, and Hepatitis C. The paper-based diagnostic kits are also used for environmental monitoring and testing food quality.

Like the litmus paper test, the disease diagnostic paper-strip kits are also pretty straightforward to use. They are equipment-free devices that display visual indicators for results.

However, the development of a paper-strip diagnostic kit itself is not easy. Considerable research goes into developing such a kit. Then there are lots of real-time testing which is then followed by bulk manufacturing of the kits. Finally, it is made available at points-of-care such as hospitals, health workers and even directly into the hands of the users as in the case of the pregnancy kits.

The paper-based diagnostic kits are lightweight and compact, making it easy for bulk transportability, have low manufacturing costs, and importantly, allow risk-free disposal by incineration. These and other factors make paper-based kits a valuable disease diagnostic tool, particularly during an epidemic or pandemic like COVID-19. In fact, the World Health Organization (WHO) has set out the principles for diagnostic kits, particularly those that are required in resource constraint environments. Irrespective of what the diagnostic kits are made of, the kits have to conform to the 'ASSURED' principle, that is the diagnostic kits have to be Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment free and deliverable to end-users Evidently, the ASSURED principle perfectly fits paper-based diagnostics.

So, how do these paper-based kits work? They work on the principle of microfluidics – which has to with the behaviour of fluids through micro-channels. So, as imagined correctly, a droplet of liquid on the paper percolates through the micro-channels in the paper. Along the way, it reacts with the chemicals, proteins, antibodies, or biomolecules that the paper has been previously smeared or coated with, changing colour owing to the reaction and to yielding a visible result.

For example, in the case of the litmus paper, there is the lateral flow of the droplet through the micro-channels in the paper strip that contains anthocyanin. This naturally occurring pigment reacts with the acidic or alkaline solution and changes colour. The pregnancy paper kit also works on the principle of lateral flow.

Then there are the bit more complicated, multi-dimensional Microfluidic Paper-based Analytical Devices, referred to as μPADs. In these multi-dimensional μPADs, multi-channels are created within a paper substrate. A droplet instead of laterally flowing all across the paper, in μPADs its flow is split into multiple streams with each stream leading to a different reactant. The other types of multi-dimensional μPADs are crafted by stacking or by folding the paper-strips, thus forming a multiple-layer microfluidic which could be used for more sophisticated testing such as those of mRNAs.

Paper as a microfluidic device is preferred because of the hydrophilic nature of cellulose, making up the paper, which channelises the liquid to places on the paper where the chemical or biochemical reactions take place.

The fluidic channels in the multi-dimensional μPADs are engraved using wax printing that guides the fluids through the paper that now has hydrophilic and hydrophobic zones. Well-known traditional printing techniques such as inkjet, screen and flexographic printing are used for etching the channels on the paper.

Paper as a microfluidic device is preferred because of the hydrophilic nature of cellulose, making up the paper, which channelises the liquid to places on the paper where the chemical or biochemical reactions take place. Now, the paper-strip based pregnancy kit readily available across the counter is not cellulose, but nitrocellulose-based kit.

In the pregnancy test, when a droplet of urine is placed on the strip, a complex process is set in motion. There is an absorbent material that filters out any bacteria and proteins present in the urine droplet. What remains is mostly water and an antigen which makes its way to a nitrocellulose paper that has been treated or coated with antibodies. In simplistic terms, the antigen-antibody binding occurs to visually indicate the results.

Then there is the nanocellulose which in recent years, apart from its other biomedical applications, has been receiving some attention as a diagnostic device as well. Nanocellulose or cellulose nano-fibres as it is called is also extracted from cellulose. Other advancements in paper-based kits include mounting electronic components including different kinds of sensors and displays to enhance the sensitivity and accuracy of the kits.

Paper-based kits do have some limitations though. The visual results yielded are not quantitative. And shelf-life of reagents on the strips and the fluidic control is also a challenge. Also, many paper-based devices require complicated manufacturing procedures that must be simplified before commercialisation.

In these challenging times, CSIR-IGIB's development of the inexpensive COVID-19 testing kit is timely and can save lives.

Dr G. Mahesh is Senior Principal Scientist, CSIR-NISCAIR