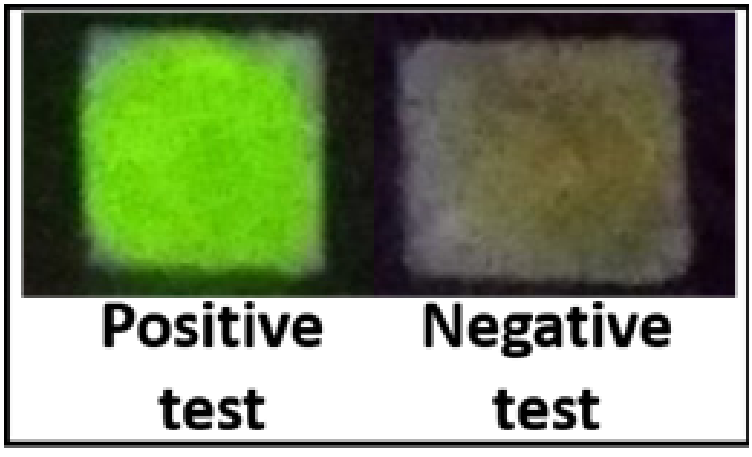
Paper Machines for Rapid and Inexpensive, Point-of-Care Diagnosis of Tuberculosis

Navjot Kaur*
Indian Institute of Science (IISc), Bangalore
Email: kaur19.navjot@gmail.com

India is one of the six high-burden countries of the world in terms of Tuberculosis (TB) load and accounted for 26 per cent of global deaths due to TB in 2016. TB is a communicable disease that spreads through air and majorly attacks human lungs. Even though the disease is curable, people still lose their lives to this painful disease, which is caused by a bacterium called Mycobacterium tuberculosis (Mtb). The treatment of the disease is also a dreadful process. The medications used for the treatment take a toll on the patient's body resulting in loss of hair, appetite and weight, anxiety issues and depression. To add to their miseries, people lose their livelihoods and become socially disconnected. Children are forced to give up school, their outdoor hobbies and stay quarantined. All this at a time when the patient badly requires financial and emotional support. The complications magnify in the current scenario, with the high prevalence of anti-microbial resistance. Many strains of the bacterium have evolved over time to evade the effect of many medications prescribed for TB treatment, which is termed as development of anti-microbial resistance. In such cases, stronger medicines are prescribed for treatment that comes with their own set of enhanced side-effects.

A major challenge in eradicating TB is the lack of appropriate diagnostic methods. The conventional treatment strategy is based on a preliminary diagnosis after which the first line medication is started. But the confirmatory results come from a bacterial culture test which takes 6 to 8 weeks. For this duration, there is no clear idea about the infection levels in the patient's body and about the type of strain infecting the patient. It might so happen that the patient is infected by

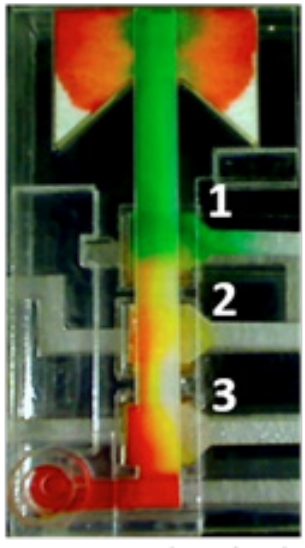
* Ms. Navjot Kaur, Ph.D. Scholar from Indian Institute of Science, Bengaluru, is pursuing her research on "Automatic Paper Machines for Rapid Detection of Tuberculosis at the Point-of-care." Her popular science story entitled "Paper Machines for Rapid and inexpensive, Point-of- care Diagnosis of Tuberculosis" has been selected for AWSAR Award.



a resistant strain and then the first line of drugs would not work. It is also possible that the patient did not have the disease but kept on taking the anti-biotics. This is one of the major reasons for the widespread development of anti-microbial resistance. A recent advancement in this direction has been the introduction of the Cepheid GeneXpert system which can provide TB test results in a couple of hours. But even after being highly subsidised by the government, a test on Cepheid GeneXpert is out of reach for people living in low resource settings; while it is these people who are highly prone to being a victim of TB owing to their poor hygiene and immunity levels. Therefore, it becomes imperative to develop diagnostic technologies that are affordable and deliverable to people living in such underprivileged areas.

We at 'The Toley laboratory for microfluidic bioengineering' at IISc, Bangalore, guided by Dr. Bhushan Toley aim at enabling rapid, robust and inexpensive diagnosis for Tuberculosis. Weare working on developing a 'paper-based' diagnostic tool that uses the DNA of Mtb as a target to confirm the presence or absence of TB.

The Cepheid GeneXpert system also uses Mtb DNA as the target but is very expensive because it employs the age-old polymerase chain reaction (PCR) for amplification of bacterial DNA. PCR is based on different temperature cycles, each cycle lasting for small intervals of time, which requires a sophisticated temperature control mechanism. We address this challenge by using a DNA identification technique which operates at a single temperature. The paper and plastic based TB testing device designed and fabricated in our lab, can be simply put in an incubator for TB testing, available even in the small labs. Fluorescent dye which forms a complex with DNA and shows a color change is used for the end-point detection. This simple detection mechanism is highly cost effective and the presence of Mtb DNA is indicated by the generation of green colored fluorescence at the end of the reaction, which takes just one hour. We envision that once produced on large scale, this will be a specific, sensitive, accessible to all, and user-friendly diagnostic tool for TB testing.



Paper to paper valves developed by Dr. Bhushan Toley. Valves 1,2 and 3 can be used for sequential delivery of different fluids.

All the biological and chemical reactions are carried out in paper-based devices as opposed to conventional tube-based reactions, because working with paper provides numerous advantages. Paper is flat, easy to engineer and can be easily designed and fabricated to suit different types of applications, without the need for specifically designed blocks or instruments required to handle tubes of different shapes and sizes. Since biological samples have limited volumes, the pore size of these porous membranes called paper, provide an appropriate sized reaction volume to carry out these biological assays. Another crucial gap that can be filled better by paper-based diagnostic devices is of the long-term storage. The low resource settings are generally miles away from a well-equipped laboratory and a pharmacy. It becomes very important that the diagnostic tools can reach to such places, at the point-of-care. Paper provides a substrate for the dry storage of the reaction reagents into the membranes which can be hydrated by the sample at the point-of-care to perform the test. We have been able to successfully conduct TB testing with reagents dried in paper, without any significant loss in efficiency.

Paper-based devices also show the potential to integrate the various steps involved in testing, which makes them highly suitable for development of point-of-care diagnostic devices. It is these point-of-care diagnostic solutions which will extend the reach of modern diagnostics to weaker economic sections. The current testing is done in a resource intensive clinical laboratory with expensive instruments and trained technicians. The patient sample undergoes various processing steps to remove the unwanted biological material and identify the intended target. If all this technology is to be taken to people sitting miles away from cities, with insufficient resources, the replacement must be automated to the maximum extent to make it independent of requirement of trained personnel. Paper to paper valves enable sequential delivery of fluids and automation of multiple user steps currently in use for diagnostic tests. While all these transformations are being made, cost is a significant governing factor. With this motivation, we are working on the development of cost-effective integration techniques to combine the various processes involved in biological testing, starting from sample preparation to obtaining the final result.

Tuberculosis is a huge national challenge and has many layers of associated problems to be addressed. With my research, I aim at providing the benefits of modern diagnostics to TB patients across economic barriers. Since the percentage of patients who discontinue TB treatment before completion is very high, rapid and specific testing can help doctors start with the right treatment in the initial phase of the disease. This will help in better management of the patients and will

also prevent the spread of the disease to other people who come in contact with the patient. A specific test will also ensure that only people who are truly infected will take medications and play a crucial role in reducing the possibilities of development of anti-microbial resistance. In cases of epidemics, availability of these quick and portable testing methods can save numerous lives in the field, helping doctors to take faster and more informed decisions. Successful commercialization of this research will mean that we would be far better equipped to combat the most deadly infectious disease of the world.