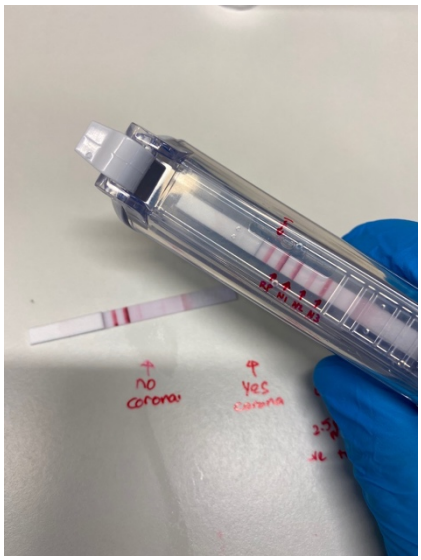
Nucleic acid-based Lateral flow (NALF) assay for COVID 19 multiplex PCR assay

Background

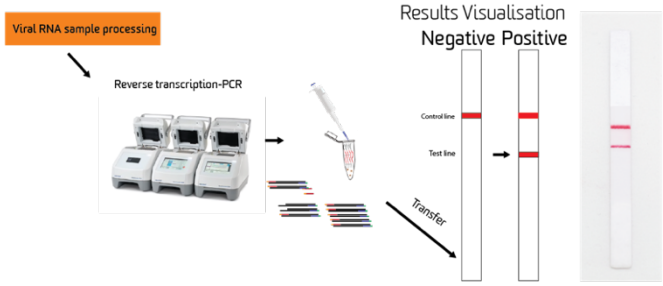


The COVID-19 outbreak has caused a massive spike in demand for molecular diagnostic testing capacity, and it is anticipated that demand for testing in parts of the world will continue to outstrip supply. Contrasting the situation of Italy and South Korea, we have seen South Korea demonstrating the efficacy of extensive deployment of testing curbing the disease spread since the Daegu outbreak in February 2020, while Italy has struggled with the uncontained spread of COVID-19. While not every nation would share the same capability in infectious disease management, countries with high throughput testing capability enable first responders to be mobilised quickly in contact tracing and isolating infected patients, preventing these cases becoming clusters which lead to uncontrolled community transmission. Countries without such resources are more likely to struggle with disease containment.

Despite the recent advances in rapid molecular diagnostic tools demonstrated in the literature to date, a versatile, widely deployed rapid point-of-care diagnostic system remains a distant reality. The COVID-19 outbreak highlights the gap and significant challenge in rapidly certifying a new diagnostic platform or test for medical diagnostics use unless exempted by Emergency Use Authorisation. Established laboratory-based diagnostic infrastructure (for viral infections) is overwhelmingly reliant on polymerase chain reaction (PCR) or alternative nucleic acid amplification protocols. PCR has been an integral tool in Nucleic Acid Amplification Testing (NAAT) in the current healthcare system for three decades, with kits for first-line diagnostics designed for ease of implementation with minimal training or change in procedure certified by peak bodies (e.g. FDA/CE-IVD).

Current Development at CERRF

The current gold standard method for the new coronavirus SARS-CoV-2 diagnosis relies on the use of real-time polymerase chain reaction (RT-PCR) instruments to determine the viral loads from clinical specimens. At Deakin, CeRRF researchers have adapted the USA CDC SARS-CoV-2 Real-Time RT-PCR Diagnostic Panel to their Nucleic Acid lateral-flow assay (NALF) to demonstrate the detection of SARS-CoV-2 as part of a multiplex test panel. The NALF operates in a comparable manner as a home pregnancy test, where a PCR amplified sample (here containing amplified nucleic acids sample) is placed on a test strip and the status of the sample (i.e. positive or negative) is indicated by bands that develop on the strip. The method works in conjunction with conventional PCR machines, and the workflow builds on the existing diagnostic workflow and capability in clinical sample handling (viral RNA purification) and PCR protocols but provides a direct visual readout after COVID-19 panel amplification.



This proof of concept platform for qualitative endpoint detection could potentially serve as an adjunct testing panel candidate to improve screening capacity at central, or remote testing laboratory's or to ameliorate backlogs by better utilising of pre-existing NAAT facility, especially in the low resource settings where in some instances access to real-time PCR instruments can be limited.

At the time of writing, the WHO website lists seven COVID-19 interim molecular diagnostic assays with the majority of them being simplex panel analysis. By leveraging the unique spatial multiplexing ability of NALF, CERRF researchers improve the US CDC COVID-19 panel testing throughput by combining the three required primer sets into a single (triplex) assay (i.e. eliminating the need for three separate analyses). A similar strategy for Charité, Berlin panel is currently underway. This has the potential to dramatically increase testing capability, particularly in low resource situations such as developing countries or in rural and regional contexts.