The Quest for a Cure

It's of little doubt that there is a great need for the development of rapid diagnostic tests to tackle the COVID-19 pandemic

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In 2003, the outbreak of severe acute respiratory syndrome (SARS) became the first pandemic of the 21st century and exemplified the rapid rate at which a virus can spread internationally. Originating in China, SARS quickly spread to other Asian countries, in addition to confirmed cases outside of the continent, there were four cases in the UK, and a significant outbreak in Toronto, Canada (1-2). Since then, several viral outbreaks have occurred globally, including the Zika epidemic, occurring in South and North America between 2015-2016, and the COVID-19 (SARS-CoV-2) pandemic 2019-2020. In comparison to SARS (over 8,000 cases), and Zika (approximately 1.5 million cases), the current COVID-19 outbreak has over 4.9 million cases, with 248,297 total laboratory-confirmed cases in the UK alone at the time of writing (2-4). The rapid growth at which this virus has spread internationally exemplifies the urgent and increasing need for rapid development of readily available, high-performing diagnostic tools, to support global efforts in controlling pandemics.

As stated by WHO, diagnostic testing for COVID-19 is critical to track the virus, understand epidemiology, inform case management, and suppress transmission (5). With COVID-19 symptoms not easily distinguishable from the common cold or flu, rapid population screening paired with lab diagnostic testing is one of the most effective methods to control the spread of infection, enabling earlier quarantine and treatment. However, for this to be effective, diagnostic developers must evaluate and manufacture tests as quickly as possible while we are still learning about the emerging virus, and be able to quickly adapt to changes in the outbreak, to best prepare for reoccurrences.

Traditional Lab Testing

Diagnostic testing methods for an infectious disease harness a variety of technologies, including molecular and immunoassay platforms. Once an infectious disease outbreak is known, polymerase chain reaction (PCR) molecular testing is the frontline response as the required primers can quickly be developed and implemented once the viral sequence is known (6). PCR enables the amplification of a small sample of DNA to accurately detect the presence of viral RNA or the viral genome early on in infection, rather than detecting antibodies, the body's immune response, which take longer to present. An additional step is required when using PCR to test for RNA viruses, such as SARS-CoV-2, to enable the conversion of viral RNA to a complementary DNA template via reverse transcription-PCR (7). Despite its high levels of

sensitivity and ability to test accurately using only a small sample of viral RNA, PCR testing requires high quality swab specimens and expensive facilities, with highly trained specialised technicians to run the tests. Therefore, PCR testing is limited to use in centralised diagnostic labs, which have quickly reached maximum capacity (6, 8). While the PCR test itself typically takes four to six hours to complete, the total turnaround time on results is approximately 24 hours (6).

In the midst of the COVID-19 pandemic, diagnostics developers are also looking to utilise alternative platforms to increase testing capabilities, particularly those already commonly found in labs.

Immunoassays can be used to test for the presence of a specific viral antigen, or specific antibody formed in the body's response to the infection. As antibodies are produced one to two weeks after infection, these tests identify patients who have been infected previously and, therefore, should be immune (though, SARS-CoV-2's immunity period has not yet been determined) (9). The enzyme-linked immunoassay (ELISA) is a common immunoassay used in central labs, enabling automation and high-throughput processing of samples. ELISA technology harnesses the highly specific binding event that occurs between an antigen and antibody to provide quantitative bioanalysis of the presence of specific biomarkers. However, as with PCR, the ELISA platform's complexity and associated dependence on highly trained users makes it unsuitable for use outside of a lab, or to provide patients with rapid test results (10).

The Use of Antibodies in Immunoassay Diagnostics

Although antibodies have shown to be effective reagents across biomedical research and immunoassay formats, they have well-documented limitations, including high cost, long development and manufacturing timelines, and poor specificity against certain targets, with some targets too difficult to generate antibodies for using traditional processes (11-12). The timeline of test development and evaluation can therefore be prolonged when relying on the use of antibodies, which in turn can restrict the ability of diagnostics companies relying on antibodies to respond to outbreaks fast enough.

In recent years, there has been a push to explore recombinant antibody alternatives to offer performance and cost improvements across diagnostic platforms, including features such as high target specificity and selectivity within a variety of matrices, consistent batch-to-batch performances, and shorter development lead times. By overcoming some of the major limitations associated with antibodies, non-antibody affinity reagents, such as adnectins, affibodies, Affimers®, anticalins, DARPins, fynomers, and kunitz domains, support the faster development of improved diagnostic tools, to better meet the validation criteria for regulatory approval and timely application in outbreaks.

Rapid POC Testing

Point-of-care (POC) or near-patient testing offers a critical solution in cases of pandemics for effective mass-monitoring and disease management, by enabling rapid detection of the virus without the need for specialised lab equipment. Whilst POC devices often require use by a trained healthcare professional, testing can be carried out at bedside with no need for samples to be transported to a lab, freeing up capacity at testing facilities in central hospital labs, such as PCR and ELISA platforms. The introduction of POC testing reduces turnaround time to 30-90 minutes generally, depending on the system, how many samples it can run simultaneously, and how many instruments are available.

POC testing devices generally fall into two main categories: larger devices that harness molecular testing techniques, or smaller hand-held immunoassay devices (13). The larger devices have overlapping technologies with those used in the central lab, miniaturised for use inside and outside of hospital environments. For rapid results, molecular test developers must provide real-time amplification of the sample, for example, via real-time PCR; however, this process requires thermocycling equipment, and is, therefore, inappropriate for some environments across developing countries requiring access to POC devices. To combat this, various enzyme-free and isothermal amplification techniques, including loop-mediated isothermal amplification and circle amplification (RCA), have been developed (13-14).

Lateral flow assays (LFA) are a widely used immunoassay for POC diagnostics due to their ability to combine rapid results with cost-effective testing devices, achievable by non-specialists. An LFA test strip is very simple; involving the chromatographic separation of a test solution across a nitrocellulose membrane and the identification of a specific analyte by binding to affinity reagents on the test strip to give a signal (15).

Consumer Testing

In contrast to typical POC testing, consumer, or self-tests, are almost all immunoassay based, mostly LFA, and can take as little as 10 minutes to give results. The most well-known application being pregnancy tests – the Clearblue test, in 1988, was the first commercial application of lateral flow technology. Consumer devices can be used by a layperson, usually outside of a healthcare setting, and without medical support. While both POC and consumer tests are key to low-resource communities that do not have access to sufficient lab testing facilities, consumer tests are particularly important to vulnerable groups, such as immunocompromised patients. Enabling testing away from high-risk environments, such as community centres and clinics, not only prevents susceptible groups from coming into contact with the virus, it also allows potential carriers of the virus to remain isolated and avoid its further spread. Recognised as the main tool to enable

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mass population testing, the consumer test is the one with the largest available market: to use the COVID-19 pandemic as an example, almost everyone can be tested, at least once, with access to a self-test, which is of particular interest to many employers to support employees' safe return to work so that the global economy can be restarted.

Meeting Demand with High Performance Diagnostic Tools

Before a POC or consumer test can be recommended by a government, the device must be validated in the appropriate populations and settings (16). This prevents inadequate or inaccurate testing that would impede disease control efforts, and can, therefore, have a longer development process than standard lab diagnostic tests, with only a subset meeting the necessary levels of accuracy. It is therefore necessary to efficiently utilise lab facilities, where tests are more quickly customised to the virus, while awaiting the development and mass manufacture of POC and consumer diagnostic devices.

In response to the COVID-19 pandemic, governments globally are strategically scaling-up diagnostic development programmes, to urgently provide testing for those on the frontline, and for all who need it long-term. To achieve this, a combinatory approach of increased POC and consumer testing for population screening in parallel with lab-based diagnostics is required; thereby supporting healthcare systems and reducing the need for mass lab-based testing.

Furthermore, rapid international spread of the SARS-COV-2 virus has increased the geographical area where testing needs to be implemented, with initial intensified molecular testing leading to shortages of testing reagents globally (17). We can draw comparisons from previous outbreaks, such as SARS, to make predictions on the possible trajectory of the COVID-19 pandemic, for example, the likelihood of a second, smaller peak of new cases (18). It is, therefore, increasingly important to explore alternative methods, systems, and reagents for accurate diagnostics to increase testing capabilities, restrict the virus' further spread, and vitally, protect the more vulnerable communities that may be less equipped to tackle and recover from the outbreak.

References

1. *Visit: www.nhs.uk/conditions/sars*
2. *LeDuc JW and Barry MA, SARS, the First Pandemic of the 21st Century,* Emerg Infect Dis *10(11): p26, 2004*
3. *Visit: apps.who.int/iris/bitstream/handle/10665/204348/zikasitrep_5Feb2016_eng.pdf;jsessionid=F386B75456CB07339F48BD7203664766?sequence=1*
4. *Visit: covid19.who.int/?gclid=CjwKCAjw8J32BRBCEiwApQEKgbWRRU6gd2I1pB4gWcsliszjF-IV1_vkoJyTqyC2XuPHojMOW7yDzRoCT4YQAvD_BwE*
5. *Visit: www.who.int/docs/default-source/coronaviruse/situation-reports/20200323-sitrep-63-covid-19.pdf?sfvrsn=b617302d_4*
6. *Visit: www.nature.com/articles/d41587-020-00010-2*
7. *Visit: thenativeantigencompany.com/why-we-need-antigen-and-antibody-tests-for-covid-19*
8. *Visit: europepmc.org/article/ppr/ppr116975*
9. *Visit: www.medicaldevice-network.com/features/types-of-covid-19-test-antibody-pcr-antigen*
10. *Kozel TR and Burnham-Marusich AR, Point-of-care testing for infectious diseases: past, present, and future,* J Clin Microbiol *55(8): pp2,313-20, 2017*
11. *Chames P* et al, *Therapeutic antibodies: successes, limitations and hopes for the future,* Br J Pharmacol *157(2): pp220-33, 2009*
12. *Visit: www.nature.com/news/reproducibility-crisis-blame-it-on-the-antibodies-1.17586*
13. *St John A and Price CP, Existing and emerging technologies for point-of-care testing,* Clin Biochem Rev *35(3): pp155-67, 2014*
14. *Hartman MR* et al, *Point-of-care nucleic acid detection using nanotechnology,* Nanoscale *5(21): pp10,141-54, 2013*
15. *Visit: avacta.com/how-a-lateral-flow-rapid-test-works*
16. *Visit: www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19*
17. *Visit: apps.who.int/iris/bitstream/handle/10665/331509/WHO-COVID-19-lab_testing-2020.1-eng.pdf?sequence=1&isAllowed=y*
18. *Hung LS, The SARS epidemic in Hong Kong: what lessons have we learned?* J R Soc Med *96(8): pp374-8, 2003*

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