With Prime Minister Boris Johnson promising the UK a lockdown-free Christmas, how can we ensure that we keep family, friends and the most vulnerable populations safe this holiday season? Point-of-care (POC) testing will play a critical role, with POC testing for SARS-CoV-2 now a part of normal life for many.

Testing for SARS-CoV-2 has become the very first point of call for those exhibiting symptoms of respiratory illness; however, could it now be just as important to test for other respiratory viruses to ensure a rapid and accurate diagnosis? Due to multiple lockdowns and other COVID-19 restrictions over the past 18 months, there is also now fear that a lack of exposure to bacteria and viruses outside the home could have caused weaker immune systems, especially in babies and young children with developing immune systems.

In the UK this past fall, many of the population have experienced what has been termed ‘the super cold,’ with the UK government also urging the public to get their flu vaccinations to go some way towards mitigating potentially overloaded hospitals. As of 22 November 2021, over 4 million people have had their flu vaccination in the UK [1].

Although COVID-19 cases have been on the rise in the UK over the past few weeks, the number of people infected has not exceeded the level that would send the UK into Plan B. At the time of writing (29 November 2021), the UK government has resisted calls to make people to work from home if possible; however, they have made mask wearing compulsory on public transport and in shops, and will be offering all over-18s booster vaccinations.

However, this is not the same for the rest of the globe, as countries such as Austria are currently experiencing lockdowns for the unvaccinated after many people decided against having the vaccination. The Austrian government are pushing to bring in mandatory vaccinations for the whole country, and whether this approach is ethical is a different story entirely.

In this Technology News, we’ll explore the brief history of COVID-19 testing, compare the different tests available on the market, look at current research into new types of POC testing for COVID-19 and other respiratory viruses, as well as discover the additional impacts of the pandemic outside of COVID-19.

POC TESTING FOR COVID-19
From the very beginning of the COVID-19 pandemic, it was immediately clear that there was a vital need for POC tests for early disease detection, diagnosis and maintenance. These tests needed to be cheap, simple to operate and have a rapid turnaround time. On 23 December 2020, the UK’s regulator (the Medicines and Healthcare Products Regulatory Agency – or MHRA) approved the Innova lateral flow COVID-19 test for people to self-administer in a social care setting. Then, on 9 April 2021, the government announced free twice-weekly rapid testing available to everyone in England. There were, however, concerns over the accuracy of the tests and the false sense of security it may have provided people with, as lateral flow tests have a lower sensitivity compared with PCR tests [2,3].

Lateral flow tests contain antibodies that recognize antigens in biological samples such as saliva. The sample is placed on a paper strip coated in antibodies, which bind to SARS-CoV-2 proteins and change color if the proteins are present. However, lateral flow tests cannot detect low levels of SARS-CoV-2 proteins and there may be a negative result for those who have only recently been infected with the virus. Data from a mass testing program in Liverpool (UK) found that lateral flow tests detected 48.89% of COVID-19 infections in asymptomatic people, compared with PCR tests.

PCR tests are the current ‘gold standard’ for the confirmation of SARS-CoV-2 infection. The technology works by screening for the presence of viral RNA, which is detectable in the body before antibodies and symptoms of the disease are present.
However, PCR tests have to be sent off to a laboratory for analysis, meaning that it can take a day or so to receive results from the test.

Other methods of POC testing include loop-mediated isothermal amplification (LAMP) processes, such as reverse LAMP and RT-LAMP. In an article recently published in BioTechniques, the authors describe a method for the rapid visual detection of SARS-CoV-2 by colorimetric LAMP. A saliva sample is added to a solution containing two primers, which then changes color in the presence of SARS-CoV-2 RNA in 16 min – this procedure could be a sensitive, alternative rapid POC test for SARS-CoV-2 RNA visual detection [4].

For many people, lateral flow testing has become part of a weekly testing routine for the workplace, when attending large venues, meeting up with vulnerable people and to afford peace of mind before mixing with others. The development of rapid POC tests that have a high sensitivity and specificity comparable to the current gold standard techniques can significantly aid testing expansion [5]. It is important to maintain regular testing routines to help stop the spread of the virus and emerging variants.

**REWRITING THE COVID-19 POC BRIEF HISTORY BOOKS?**

Lateral flow tests have now become a routine part of life in the UK; however, the sensitivity and accuracy of these tests still leave vast room for improvement. A technology that could fill this gap is Oxford Nanopore’s (UK) LamPORE, which combines LAMP and nanopore sequencing.

In a clinical evaluation of people with no COVID-19 symptoms, LamPORE had a sensitivity of 99.57% and a specificity of 99.4%. They are much more accurate than the current lateral flow tests being used for monitoring the asymptomatic population in the UK, can use saliva as well as swabs, and positive results do not require a confirmatory PCR [6].

LamPORE is currently CE marked for in vitro diagnostic use and Oxford Nanopore have expressed their intent to submit to regulatory bodies for diagnostic use approval in other countries, including with the US FDA [7]. The UK government in fact signed a deal with Oxford Nanopore in August 2020 [8] to provide rapid LamPORE COVID-19 testing to hospitals, with mobile pop-up pilot laboratories implemented in four locations in January 2021. However, the UK government ended this contract early in mid-August 2021 after deciding that there was no longer a need for LamPORE COVID-19 testing. Although LamPORE is more mobile than the current ‘gold standard’ PCR testing laboratory and can turn around results quicker, could the convenience of lateral flow tests have won out in the UK population? All is not lost for Oxford Nanopore though, as it’s believed around one-in-five COVID-19 tests globally make use of their genome sequencing tools [10].

**DIAGNOSIS THROUGH MULTIPLEXING**

The LamPORE assay has concurrently been developed to also detect other respiratory viruses to a high accuracy [10], which is of particular importance this winter while cases of additional respiratory viruses, such as influenza and respiratory syncytial virus (RSV), continue to rise.

One of the primary advantages of LamPORE testing is its throughput capabilities, which stands at 768 samples per flow cell, in comparison to 96 samples per plate in traditional testing [11]. This enables LamPORE to be leveraged for enhanced multiplexing – meaning that it can screen samples for multiple respiratory illnesses at the same time as screening for SARS-CoV-2.

COVID-19 POC testing has now become commonplace to diagnose, inform clinical and personal decisions and go some way towards preventing mortality. The benefits of multiplex testing could be enormous – patients could rapidly be correctly diagnosed and provided with the correct treatment in a matter of hours, which could be the difference between life and death in some cases.

Prior to the COVID-19 pandemic, influenza was causing 11,300 deaths per year on average in England [12], in part due to a lack of diagnosis as routine POC testing for respiratory illnesses was practically nonexistent. Due to the implementation of routine POC testing for COVID-19 and the successes that have been proven in accurate diagnosis for disease management and treatment, the question now remaining is should POC testing for other respiratory illnesses be more commonplace to prevent mortality, as has been the case for COVID-19? If so, it seems multiplex testing could be the way to achieve this.

**STAGNANT LOCKDOWNS LEAD TO LEGIONS OF BACTERIA**

While the rapid development of POC testing over the last 2 years has also improved diagnostic capabilities for further respiratory infections, the COVID-19 pandemic has had further wide-ranging impacts on patterns of infection for other diseases.

A particularly prominent example of this is Legionnaires’ disease, caused by the water-borne bacteria *Legionella* spp., which flourishes in stagnant water. The bacteria infect the lungs, inducing a pneumonia that can be fatal in 10% of cases. Reported cases of Legionnaires’ have been dramatically increasing over the last decade [13], in part due to improved awareness and diagnosis of the disease, but also due to climate change, increased travel and increasingly elderly populations, who are more vulnerable to Legionnaires’.

Lockdowns around the world have increased the number of buildings standing empty and unused around the world, creating the ideal environments for *Legionella* spp. to multiply. Many are concerned by these circumstances and reports around the world are already indicating that Legionnaires’ outbreaks are occurring at a higher rate than normal [14].

In a recent interview with BioTechniques, Orla O’Connor & Elizabeth Minogue of BioProbe Diagnostics, a spin-off company from the National University of Ireland (Galway, Ireland), exposed the issues with current testing for Legionnaires’ [15]. Current testing involves lengthy cell culture steps that delay test results for up to 14 days. They then highlighted the need for improved testing for Legionnaires’, closer to POC diagnostics:

“...this [Legionnaires’ fatality rate] rises to 25% when infection is associated with healthcare-associated infections (HCAIs),” they stated, going on to emphasize that “Timely identification
and reporting of Legionella is critical in reducing infection rates ... particularly in hospital settings, where vulnerable patients, such as older people or those with pre-existing health conditions, are most susceptible."

To address the need to detect this bacterium as soon as it becomes prevalent, BioProbe Diagnostics has developed a test kit that can conduct DNA extraction and purification followed by a real-time PCR assay to deliver test results in less than 5 h. While the kit still requires the test to be completed in a laboratory, this comes pretty close to providing a POC screening system for a building’s water system for hospitals that often have on-site laboratories.

**ENTERIC PATHOGENS RE-ENTER SOCIETY**

Enteric diseases have also been impacted by the COVID-19 pandemic. Recently, a paper in *The Lancet* reported decreases in the test positive results for adenovirus (-72%), norovirus (-59%), rotavirus A (-54%) and more during the first year of the pandemic [16]. This was reflected by a decrease in enteric bacteria test positive rates as well, excluding nontyphoidal *Salmonella* and *Campylobacter coli*.

The researchers of this study predicted that as non-pharmaceutical interventions, such as social distancing and mask wearing, were repealed, a resurgence in these enteric pathogens will result as populations will have lower levels of immunity. These predictions have already been proven accurate in the UK, where outbreaks of norovirus – typically a virus that is most prevalent in the winter – were three times more common than usual during the summer months [17]. Enteric virus outbreaks as a whole were also seen to increase over twofold in the short period of time between 31 May and 4 July 2021 [17].

These figures are mirrored in the USA, where the percent positive rates for norovirus skyrocketed from 2.4% at the beginning of 2021, to a peak of 12.4% in March (Figure 1) [18]. Although rates have since decreased, the test positive percentage remains significantly higher than comparable periods in 2020.

During the winter months, healthcare settings experience additional pressures exerted by the cold conditions and the induction of ideal environments for the spread of disease. As a series of different diseases with symptoms that could be mistaken for COVID-19 are rising in prevalence, the need for POC diagnostics that can determine if a patient is infected with an enteric disease, COVID-19, the flu or Legionnaires’ becomes clear.

To distinguish between these conditions quickly and accurately, allowing clinicians to pursue the best course of treatment for each patient, will be vital to efficiently treat the vast number of patients seen over the winter and, ultimately, save lives.

While the logistical and economic structures may not be in place to provide a diagnosis for each common disease outbreak, there are certainly steps that can be taken to protect the most vulnerable populations from experiencing severe disease outcomes. It seems that practicing good hygiene, distancing from others when unwell, keeping up to date with vaccinations and confirming COVID-19 infection where possible by leveraging established, widely available POC tests will be critical this holiday season.

Moving forward, with the observed fluctuations in enteric disease and Legionnaires’ infection rates in mind, it may be worth questioning if a certain level of viral and bacterial exposure is necessary to maintain the inner workings of our immune systems and to prevent diseases tearing through unprotected populations as restrictions are repealed.

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**Figure 1.** A graph plotting the percent positive rate for norovirus PCR tests in the USA between August 2020 and late July 2021. A large peak can be seen in March 2021 as certain lockdown restrictions were eased in the USA. Reprinted from National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases.
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