# Why the Development of COVID-19 Diagnostics is Far from Over

Given the need for expanded diagnostic testing, many point-of-care tests for COVID-19 have been rapidly developed for both medical and public use. However, the pace of assay development has at times exceeded rigorous evaluation, and uncertainties still remain about the accuracy and reliability of these kits. This article explains the need for rapid point-of-care (PoC) testing, issues surrounding reliability and validation, and how the choice of reagents can affect diagnostic performance.

**The Role of Diagnostics**

Since its emergence in late 2019, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has spread to over 200 countries, infecting over 12 million individuals and resulting in at least 500,000 deaths worldwide[1]. While the case fatality rate of infection is relatively low (estimated at around 1%[2]), SARS-CoV-2 has proved to be a highly capable pathogen, combining long incubation periods with high transmissibility and a substantial proportion of asymptomatic-to-mild infections that have made it highly challenging to detect and contain. As it currently stands, there are no vaccines or specific treatments available beyond a handful of investigational therapeutics that are limited to the severest cases. As a result, non-pharmaceutical interventions have become the mainstay of disease prevention, with measures including self-isolation, travel quarantines, social distancing and personal protective equipment. Despite such implementations, many countries have been caught underprepared and as a result have faced ongoing community and nosocomial transmission. To reduce transmission to manageable levels and contain further outbreaks through contact tracing, information derived from diagnostic testing will be crucial. Not only does such data allow identification, isolation and treatment of cases, but it also provides the epidemiological variables to inform ongoing changes to public health policy on both a regional and national level.

So far, the major means of SARS-CoV-2 diagnosis has been the polymerase chain reaction (PCR), a technique that amplifies small amounts of viral RNA up to detectable levels to confirm the presence of active infection. Since the early days of the pandemic, PCR has been instrumental in diagnosing cases and has shown its strengths in both speed and diagnostic sensitivity, as swabs can be sent to a clinical laboratory to provide results within a few hours and only very small amounts of RNA are needed for amplification. However, the need to detect viral RNA is also the technique's main drawback: as our bodies begin to get a hold on an infection, the virus is quickly flushed out the body and its RNA soon becomes undetectable[3]. As a result, there is only a limited time window for detection, making diagnosis of asymptomatic or sub-clinical infections particularly challenging. Another major issue lies in sample collection: as viral loads in sputum are not homogeneously distributed, there is always a chance that viral RNA is not captured, which can lead to false-negative results with potentially harmful consequences[4]. Finally, there are some practical limitations to consider: the processing of RNA samples requires specialised biocontainment laboratories, supported by complex and resource-costly sample collection and distribution systems that typically extend turnaround times beyond 24 hours[5]. Combined, these factors limit the utility of PCR and make it impractical for use at scale.

**Moving to the Point of Care**

To bolster diagnostic capacity and better track cases at the community level, more decentralised, rapid and "resource-lite" forms of testing are needed for use at the PoC. Furthermore, platforms that can detect antibodies raised to SARS-CoV-2 are highly desirable given their potential in epidemiological modelling, assigning so-called "immune passports", and in the identification of convalescent patients that can provide therapeutic plasma. While there are a range of options available, one of the best-placed technologies to meet these needs is lateral flow assays (LFAs; Figure 1). In short, LFAs are paper or polymer-based immunoassays that absorb a sample and run it along the surface of a pad, binding reporter antibodies and then detector antibodies to produce a confirmatory visual signal — usually in a matter of minutes[6]. A well-known example of an LFA is the at-home pregnancy test.

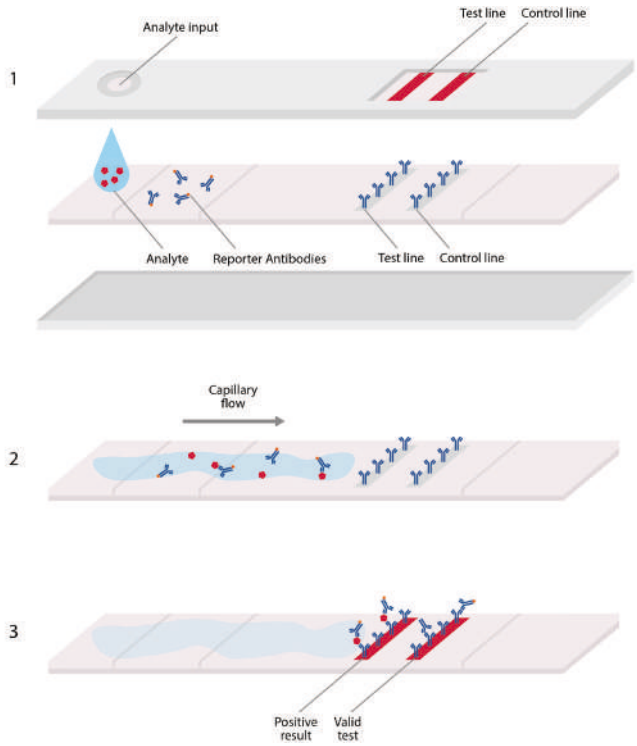


*Figure 1. Basic design of a LFA. LFAs typically comprise a plastic cassette, containing a strip of absorbent material that is able to absorb and transport an analyte. 1) Analyte is first added to one end of the strip, where it is absorbed, allowing the analyte to migrate along it. 2) Analyte encounters an area of conjugate antibodies, which adhere to them, along with detectable tags. 3) Analyte continues to migrate to an area of test antibodies that are specific to the analyte and bind to produce a solid, visible line that indicates a positive result, and hence, confirms infection. Secondary control lines are used to detect migrated conjugate antibodies and ensure that the test has worked correctly.*

A particularly useful feature of LFA platforms is their design flexibility, making them well-suited for different applications. For the detection of acute SARS-CoV-2 infection, virus or antigen can be collected from nasopharyngeal swab samples and detected by antibodies that are specific to the spike (S) and nucleoproteins (N). Alternatively, a range of different antibodies produced against

SARS-CoV-2 can also be measured from either sputum (IgA) or blood (IgM and IgG) to gauge the patient's immune status both during and long after infection. However, unlike antigens or RNA, antibodies appear unusually slowly in most COVID-19 patients, with a median time of 11 and 14 days for IgM and IgG, respectively[7] (Figure 2).

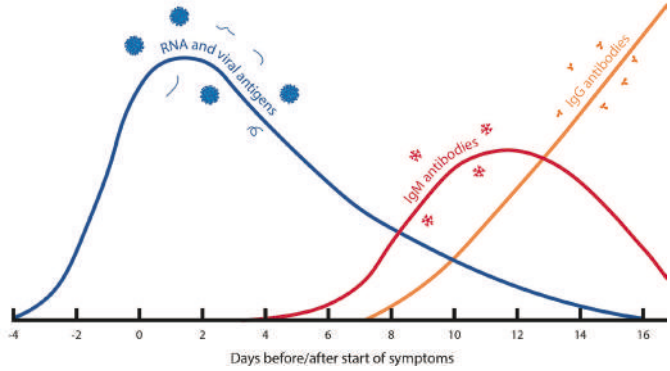


*Figure 2. Graph illustrating the general levels of RNA, antigen, IgM and IgG over the time course of a SARS-CoV-2 infection.*

Therefore, the application of antibody tests in acute-phase diagnosis is still uncertain and public health agencies have advised against the use of LFAs in directing healthcare, instead suggesting that they are used in tandem with other diagnostic technologies or in population-level epidemiological studies[8]. On the other hand, rapid antigen tests show potential in decentralised acute phase testing, especially where access to PCR is limited. Given these complementary features, the use of both antibody and antigen LFAs in combination could provide much-needed serological data, while alleviating the pressures on public testing laboratories. The secure confirmation of antibody status would allow individuals to return to work and guide policy-makers, while acute-phase status could be used to inform isolation and treatment decisions, potentially in tandem with digital approaches to contract tracing.

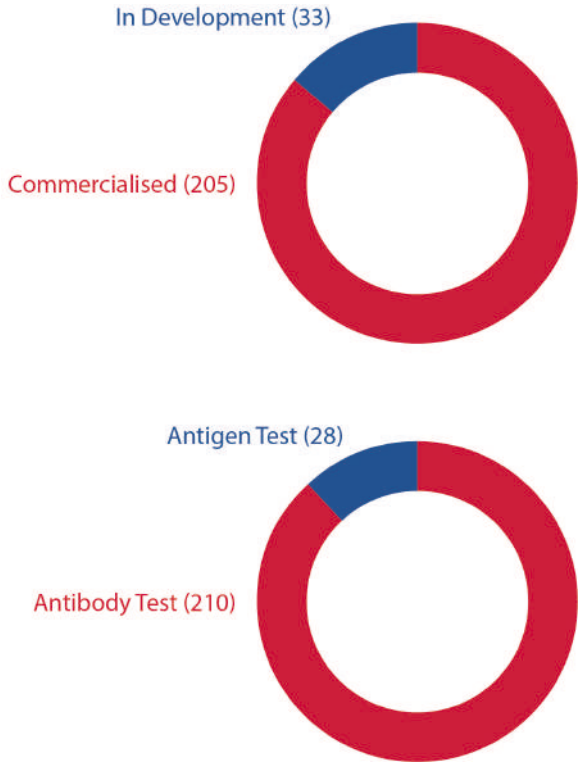


*Figure 3. Pie charts showing the proportion of rapid tests that are in development or commercialised and the proportion that are antigen- or antibody-based. Data from the Foundation for Innovative New Diagnostics (FIND)[9].*

**Prioritising Quality**

The emergence of SARS-CoV-2 and its global proliferation has spurred an unprecedented effort by diagnostic manufacturers to provide timely and effective solutions. At the time of writing, over 200 rapid tests are in development or have already been commercialised for use (Figure 3), with many being employed in small- to medium-scale serological studies[9].

However, while an abundance of tests are now available, there have been several hurdles to their effective deployment. Firstly, due to the unprecedented pace at which diagnostics have been developed, the performance characteristics of many kits have not been adequately assessed for use at the PoC. The result has been a glut of low-quality diagnostics that could potentially endanger patients, waste scarce resources and compromise public trust in healthcare services. To complicate matters, the few studies assessing the performance of such tests have showed high risks of bias and heterogeneity in evaluation standards[10], with further clinical investigations tending to show less favourable performance, and some tests having even been identified to have "fraudulent documentation, incomplete technical files or unsubstantiated claims"[11]. Finally, in the case of antibody tests, there is also still an incomplete understanding of antibody kinetics and correlates of immune protection, which limit the utility of LFAs in this application[12]. To remedy these problems, further research and assay validation are a clear priority. In particular, studies are needed in prospective cohorts for the intended use populations that include a range of ages and ethnicities, with transparent reporting of data.

**The Role of Reagents**

While LFAs are seemingly simple devices, their development is deceptively complex. The design, optimisation and validation of an assay can take years at a time and developers will often continue improving performance characteristics after initial approval to decrease the risk of false positive and negative results. Central to an assay's performance is the development, selection and application of high-quality biological reagents. Nearly all immunoassays use recombinant proteins expressed from cell culture, which offer the advantage of improved biosafety and batch-to-batch consistency (13). For COVID-19, there are two antigens that nearly all tests are based on: the SARS-CoV-2 S and N.

The S protein (Figure 4) is found as a trimer that protrudes from the surface of SARS-CoV-2 and gives it its characteristic crown-like appearance. In addition to its three polypeptide chains, each trimer contains up to 66 glycan sugars that are post-translationally added to mediate various functions during infection[14].

From the perspective of assay development, these glycans constitute many of the key surface epitopes that are recognised by host antibodies, and as a result, the use of unglycosylated S proteins risks the binding of non-specific, cross-reactive antibodies that reduce diagnostic specificity. To ensure that recombinant S protein is produced with full glycosylation patterns and proper conformational folding, developers must therefore take care in selecting and optimising their expression systems. More simplistic organisms like E. coli, for example, do not have the necessary cell machinery to glycosylate recombinant antigens, requiring more advanced systems such as mammalian or insect cell lines. When scaling-up protein production, factors such as yield and batch-to-batch consistency also require careful consideration. To further improve specificity, many manufacturers are also using select regions of the S protein which show greater

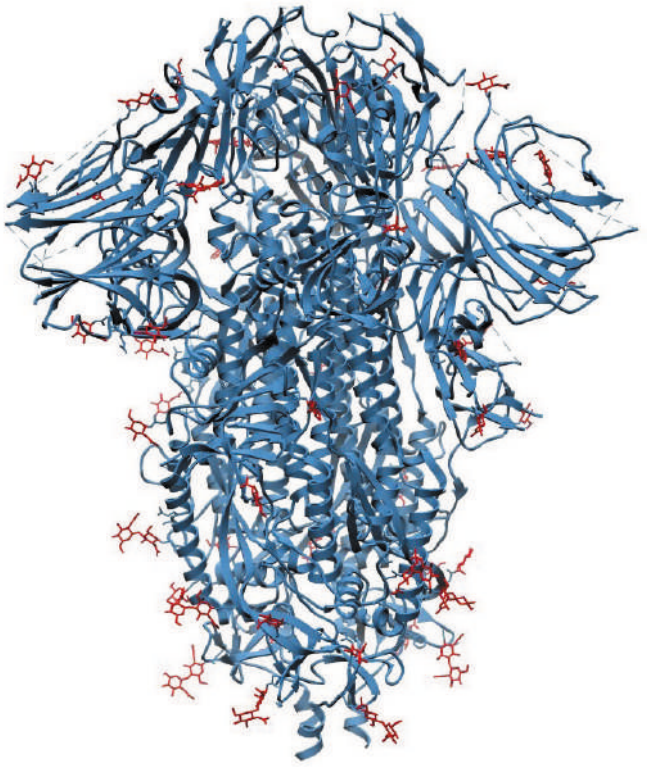

*Figure 4. 3D crystal structure of SARS-CoV-2 S protein trimer. Glycans are coloured red; amino acids are coloured blue. Structure determined by Walls et al.[15]. RCSB#: 6VXX.*

genetic variation from other coronaviruses and are therefore less able to bind cross-reacting antibodies. Popular choices are the S1 subunit of S and its receptor-binding domain, which is responsible for binding angiotensin converting enzyme 2 (ACE2) to mediate viral entry. However, this in turn brings forth new challenges, as producing and presenting these truncated proteins in their native conformation is no easy feat.

For assays that use N, the challenges are somewhat different. N is not present at the surface of the virus but are instead found within the viral capsid where they bind to genomic RNA. Unlike S, N is completely unglycosylated and shows greater genetic sequence conservation between different coronaviruses, especially at its N-terminal domain. The resulting structural similarity allows N to bind cross-reactive antibodies that have the potential to produce false-positive results. To remedy this, a popular strategy is to "ablate" cross-reactive epitopes by introducing point mutations in the N gene, while minimising structural changes to other regions. Alternatively, so-called "quenching antigens" can be introduced to "soak up" excess, cross-reacting antibodies from patient sera.

**A Balanced Approach to SARS-CoV-2 Diagnostic Testing**

Balancing the need for greater diagnostic capacity and the risk of diagnostic error remains a significant challenge to public health. To achieve the promise of widespread testing, developers must take great care in designing and validating both antigen- and antibody-based assays, with a careful consideration of the critical reagents.

**REFERENCES**


1. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. Johns Hopkins University. (2020). Available from: https://coronavirus.jhu.edu/map.html
2. Petersen, E. P., Koopmans, M., Go, U., Hamer, D. H., Petrosillo, N., Castelli, F., Storgaard, M., Al Khalili, S. and Simonsen, L. Comparing SARS-CoV-2 with SARS-CoV and influenza pandemics. The Lancet Infect. Dis. 1-7 (2020).
3. Walsh, K. A., Jordan, K., Clyne, B., Rohde, D., Drummond, L., Byrne, P., Ahern, S., Carty, P. G., O'Brien, K. K., O'Murchu, E., O'Neill, M., Smith, S. M., Ryan, M. and Harrington, P. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. Journal of Infection. 8;29, 1-15 (2020).
4. Woloshin, S., Patel, N. and Kesselheim, A. S. False Negative Tests for SARS-CoV-2 Infection — Challenges and Implications. N. Engl. J. Med. (2020).
5. Your coronavirus test result. NHS. (2020). Available from: https://www.nhs.uk/conditions/coronavirus-covid-19/testing-and-tracing/what-your-test-result-means/
6. Wu, J-L., Tseng, W-P., Lin, C-H., Lee, T-F., Chung, M-Y., Huang, C-H., Chen, S-Y., Hsueh, P-R. and Chen, S-C. Four point-of-care lateral flow immunoassays for diagnosis of COVID-19 and for assessing dynamics of antibody responses to SARS-CoV-2. J. of Infect. 22:35, 1-8. (2020).
7. Zhao, J., Yuan, Q., Wang, H., Liu, W., Liao, X., Su, Y., Wang, X., Yuan, J., Li, T., Li, J., Qian, S., Hong, C., Wang, F., Liu, Y., Wang, Z., He, Q., Li, Z., He, B., Zhang, T., Fu, Y., Ge, X., Liu, L., Zhang, J., Xia, N. and Zhang, Z. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin. Infect. Dis. 1-22. (2020).
8. Advice on the use of point-of-care immunodiagnostic tests for COVID-19. World Health Organization (WHO). Available from: https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19
9. SARS-CoV-2 Diagnostic Pipeline (Immunoassays). Foundation for Innovative New Diagnostics (FIND). (2020). Available from: https://www.finddx.org/covid-19/pipeline/?section=immunoassays#diag_tab
10. Bastos, M. L., Tavaziva, G., Abidi, S. K., Campbell, J. R., Haraoui, L-P., Johnston, J. C., Lan, Z., Law, S., MacLean, E., Trajman, A., Menzies, D., Benedetti, A. and Khan, F. A. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ. 370, 1-13. (2020).
11. An overview of the rapid test situation for COVID-19 diagnosis in the EU/EEA. European Centre for Disease Prevention and Control (ECDC). (2020). Available from: https://www.ecdc.europa.eu/en/publications-data/overview-rapid-test-situation-covid-19-diagnosis-eueea
12. Winter, A. K. and Hegde, S. T. The important role of serology for COVID-19 control. The Lancet Infect. Dis. 20:7, 758-759. (2020).
13. Intantino, M., Damiani, A. Gobbi, F. L., Grossi, V., Lari, B., Macchia, D., Casprini, P., Veneziani, F., Villalta, D., Bizzaro, N., Cappelletti, P., Fabris, M., Quartuccio, L., Benucci, M. and Manfredi, M. Serological Assays for SARS-CoV-2 Infectious Disease: Benefits, Limitations and Perspectives. Israel Medical Association Journal. 22:4. (2020).
14. Watanabe, Y., Allen, J. D., Wrapp, D., McLellelan, J. S. and Crispin, M. Site-specific glycan analysis of the SARS-CoV-2 spike. Science. 369:6501. 330-333. (2020).
15. Walls, A. C., Park, Y-J., Tortorici, M. A., Wall, A., McGuire, A. T. and Veesler, D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. PMC. 181:2. 281-292. (2020).



## Dr. Andy Lane

Andy Lane is Commercial Director at The Native Antigen Company (now part of LGC's Clinical Diagnostics Division), a manufacturer of antigens and antibodies for infectious diseases, including SARS-CoV-2. Following his PhD in pathobiology, Andy joined the NHS and led a monoclonal antibody research group working in leukaemia and lymphoma diagnostics. He subsequently joined a major monoclonal antibody supplier to lead new product development and was executive director at Innova Biosciences.

**Email: alane@thenativeantigencompany.com**