Serology testing in the COVID-19 pandemic response

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The collapse of global cooperation and a failure of international solidarity have led to many low-income and middle-income countries being denied access to molecular diagnostics in the COVID-19 pandemic response. Yet the scarcity of knowledge on the dynamics of the immune response to infection has led to hesitation on recommending the use of rapid immunodiagnostic tests, even though rapid serology tests are commercially available and scalable. On the basis of our knowledge and understanding of viral infectivity and host response, we urge countries without the capacity to do molecular testing at scale to research the use of serology tests to triage symptomatic patients in community settings, to test contacts of confirmed cases, and in situational analysis and surveillance. The WHO R&D Blue Print expert group identified eight priorities for research and development, of which the highest is to mobilise research on rapid point-of-care diagnostics for use at the community level. This research should inform control programmes of the required performance and utility of rapid serology tests, which, when applied specifically for appropriate public health measures to then be put in place, can make a huge difference.

Diagnostics: the weak link in the COVID-19 pandemic response

The COVID-19 pandemic, now only a few months old,[1,2] has brought into sharp focus inequalities within and among countries. John Nkengasong, Director of the Africa Centres for Disease Control and Prevention, reported that "the collapse of global cooperation and a failure of international solidarity have shoved Africa out of the diagnostics market".[3] Sadly, the same is true of many other low-income and middle-income countries (LMICs) outside Africa.

Why are diagnostics important? In any epidemic response, diagnostic testing plays a crucial role and this pandemic is no exception. Because early clinical presentations of infected patients are non-specific, testing is needed to confirm the diagnosis of COVID-19 in symptomatic patients, as soon as possible, so that these patients can be appropriately isolated and clinically managed.[1,4,5] Diagnostic testing is also needed for individuals who have come into contact with someone with confirmed COVID-19. Some testing strategies examine only contacts who have symptoms or develop illness of any kind during the 14-day period after contact. Other strategies examine all contacts when identified, regardless of whether they have any symptoms. Studies have shown that a large number of infected individuals might have no symptoms at all, and there is concern that these individuals are still able to shed the virus and transmit infection through saliva droplets as they speak.[4–9] Tracking all contacts of confirmed cases and testing them for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is key to successful pandemic control. Diagnostics are also needed to support rapid serosurveys that establish whether and to what extent SARS-CoV-2 has circulated in a community, and surveillance systems, such as that for influenza-like illness, that monitor disease trends over time. Diagnostics can also be used to identify at-risk populations and assess the effectiveness of control strategies.

Tedros Adhanom Ghebreyesus, Director-General of WHO, urged countries to implement a comprehensive package of measures to find, isolate, test, and treat every case, and trace every contact. Goodwill between countries has already been shown through the publishing of the SARS-CoV-2 genetic sequence and shared laboratory protocols to detect the virus.[10] However, as these molecular assays require sophisticated laboratory facilities, countries with insufficient infrastructure quickly accumulate a backlog of testing. The rapid spread of COVID-19 around the world has led to a global shortage of reagents and supplies needed for testing. Point-of-care molecular assays for SARS-CoV-2 detection are now available to enable community-based testing for COVID-19 in LMICs. Unfortunately, the production of these test cartridges takes time and, again, global demand has outstripped supply, leaving LMICs struggling for access.

Test, test, test

In March, 2020, WHO urged member states to "test, test, test".[11] Widespread testing can help countries to map the true extent of the outbreak, including identifying hot spots and at-risk populations, and monitor the rate at which the epidemic is spreading. However, most LMICs find that molecular testing, including point-of-care testing, is neither scalable nor affordable on a large scale. Relying solely on centralised testing puts countries at risk of having nothing to use. What diagnostic alternatives are available to support decentralised testing that would allow countries to mount an adequate response to the pandemic?

Rapid antigen detection tests that are simple to do at point of care and can give results in less than 30 min would be viable alternatives to molecular testing for confirming COVID-19 cases, enabling appropriate case management, and guiding public health measures, such as quarantine or self-isolation. However, although scaling up rapid antigen testing offers an effective means of triaging symptomatic individuals in community settings, early evaluations of rapid antigen detection tests show

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suboptimal sensitivity for these tests to be recommended for clinical diagnosis or triage.[12]

Rapid antibody detection lateral flow tests are also simple to use, generally requiring a few drops of whole blood from a finger prick placed onto the test strip with no processing needed. These tests take 15–20 min to do with minimal training and can be done at the point of care as most do not require any equipment. Rapid antibody testing is an attractive option for scaling up testing but only if these tests show satisfactory performance for a clearly specified use.

SARS-CoV-2 infectivity and immune response
The detection of SARS-CoV-2 infection and immune response has been described in relation to different diagnostic tests.[13] In this section, we summarise the evidence from studies to date.

Viral infectivity
Studies have shown that SARS-CoV-2 RNA can be detected 2–3 days before onset of symptoms and can remain detectable up to 25–50 days after the onset of symptoms, particularly in patients who remain symptomatic for an extended period.[7,14,15] SARS-CoV-2 RNA can be detected for longer in respiratory samples from patients with severe disease than in samples from patients with mild illness.[16] Viral RNA concentrations peak within the first 5 days after onset of symptoms and decrease slowly with rising antibody concentrations.[7,17,18] However, RNA clearance is not always associated with rising antibody concentrations, particularly in patients who were critically ill.[6,18] An important question for the potential for spread of COVID-19 is whether individuals who are RNA-positive are shedding infectious virus. A small study in nine patients found that viral replication stopped 5–7 days after onset of symptoms but patients remained RNA-positive for 1–2 weeks after this point.[6] Hence, there remains some uncertainty as to whether a patient who is RNA-positive is shedding live virus or not.

Immune response to COVID-19 disease
Maturation of the immune response typically takes 40 days with variations in the dynamics of the antibody response depending on disease severity and other factors still to be discovered. In most studies of laboratory-confirmed COVID-19 cases, IgM antibodies start to be detectable around 5–10 days after onset of symptoms and rise rapidly.[14,18–21] IgG antibody concentrations follow the IgM response closely. Seroconversion is typically within the first 3 weeks with the mean time for seroconversion being 9–11 days after onset of symptoms for total antibody, 10–12 days for IgM, and 12–14 days for IgG.[14,18,19,22]

COVID-19 antibody response: pathogenic or protective?
Antibodies against the receptor-binding domain of the spike protein and the nucleocapsid protein have been associated with neutralising activity.[14,23,24] Neutralising antibodies to these domains can be detected approximately 7 days after onset of symptoms and rise steeply over the next 2 weeks.[23,24] Several studies showed that patients can remain RNA-positive despite high concentrations of IgM and IgG antibodies against the nucleocapsid protein and the receptor-binding domain of the spike protein.[18] Whether the presence of neutralising antibodies translates into protective immunity in patients with COVID-19 is unclear. Some researchers speculate that antibodies can enhance infectivity as higher antibody concentrations have been observed in patients with severe disease than in those with mild disease.[18,25] In one study (n=222), a greater proportion of patients with high IgG concentrations had severe disease than did those with low IgG concentrations (52% vs 32%, p=0·008).[26] The role of antibody response in the pathogenesis of COVID-19 remains unclear pending further studies.

Ideal testing strategies versus reality
WHO and the Pan American Health Organization have stated that they do not currently recommend the use of immunodiagnostic tests except in research settings,[27,28] because of scarce information on test performance and appropriate use when immunity to COVID-19 is not well understood. However, many countries are struggling to scale up testing to implement the key strategies of diagnosing all symptomatic patients and tracing all contacts. Delays in confirming COVID-19 cases allow continued transmission within communities and can result in failure to contain the pandemic despite other measures such as physical distancing and travel restrictions.

How can countries move forward?
Countries are assessing all available testing options to address their range of needs. In settings where challenges with molecular testing exist or access to laboratories is scarce, rapid serology tests offer a needed additional option. A rapid serology test with good performance characteristics is extremely important to avoid missing true cases of COVID-19 and imposing unnecessary quarantine for people with false-positive results due to cross-reactivity with seasonal coronaviruses. Studies have shown more antibody cross-reactivity between the nucleocapsid proteins of SARS-CoV-2 and common coronaviruses than between their spike proteins.[20,22] Tests that use the spike protein or fragments of the spike protein as targets might have the least amount of cross-reactivity with common coronaviruses, on the basis of sequence analysis.[22] Clear articulation of the benefits and limitations of serology tests will hopefully incentivise manufacturers to improve performance.[29]

When is serology testing recommended?
Rapid triage of symptomatic individuals in community settings
Where there is little or no access to molecular testing, rapid serology tests provide a means to quickly triage

suspected cases of COVID-19, provided the test is highly specific for the disease. A positive result for IgM in symptomatic patients fulfilling the COVID-19 case definition is strongly suggestive of SARS-CoV-2 infection. This approach is probably most effective in individuals 5–10 days after symptom onset.

In Peru, public health facilities for molecular testing are sparse and only 500 beds in intensive care units exist for a population of 32 million. The Ministry of Health has set up a hotline and website for individuals who have symptoms to be interviewed by a health professional for possible follow-up, prioritising the visits according to age, risk factors, and severity of symptoms. A testing team visits the individual at home to do the rapid antibody test. Individuals who are IgM and IgG positive and have mild symptoms are quarantined, whereas people who need critical care are referred to hospital. All contacts are also tested with the rapid serology test. Anyone who tests negative in the antibody test has a swab collected for molecular testing.

As of May 2, 2020, 355 604 people had been triaged in Peru with 42 534 testing positive, 26 362 of whom were found to be positive by use of a rapid test.[30] This approach has allowed a large number of symptomatic individuals and contacts to be rapidly tested in the community, relieving the backlog, reducing waiting time for molecular testing, and preventing the health-care system from being overwhelmed.

Experience in China has also shown that, in symptomatic patients, the use of IgM tests or total antibody tests can increase the sensitivity of COVID-19 case detection.[18] Further research should explore the performance and utility of rapid antigen-IgM and antigen-IgG combo tests and the timing of testing. In individuals who test negative for IgG, research should also explore, if resources allow, the value of doing a follow-up antibody test 10–14 days later to document a definitive diagnosis through sero-conversion.

Testing all contacts of people with confirmed COVID-19

Studies have shown that a large number of infected individuals could have only mild symptoms or no symptoms at all, but they can still transmit infection, with as much as 44% of infections being transmitted by pre-symptomatic individuals.[6,7,9] Experience from Singapore shows that tracking down all contacts of people with confirmed COVID-19, testing them for evidence of infection, regardless of symptoms, and putting those contacts who test positive into isolation is an urgent priority for interrupting the chain of transmission and containing the epidemic.[31–33] This approach is particularly important in the early stages when there are only sporadic or clusters of cases, or for countries coming down from the peak to continue to reduce the extent of infection in the community. Only individuals who test negative should have a throat swab collected for molecular testing, which will reduce the strain on laboratories doing these tests.

Situational analysis and surveillance

In countries that have set up syndromic surveillance, such as surveillance for influenza-like illness or severe acute respiratory infections, and where blood or throat swabs are routinely collected at these sentinel sites, collected samples can be tested for COVID-19 with molecular, antigen, or serology tests, either alone or in combination. If any of these samples are positive, it means COVID-19 has been circulating in the community. Where serial samples are available, it might be possible to date when COVID-19 established itself in a community or country.

In general, antibody tests can be used to establish the true extent of an outbreak, map its geographical distribution, and identify hotspots and populations that are particularly at risk. This information can in turn be used to inform public health measures and control strategies. In this case, researchers need to stick to the same serology test and test sentinel populations repeatedly, avoiding the variation in sensitivity between different rapid tests.

When is serology testing not recommended?

Testing the general population

The use of serology tests for population surveys is not recommended in low prevalence settings as this approach will probably result in more false-positive than true-positive results, even if a test with high specificity is used. For example, if the prevalence of infection is 1% in the general population, a test with 98% specificity will identify two false-positive results for every true positive result. These results could lead to a false sense of security regarding the extent of immunity in the population and premature easing of public health measures on the basis of misleading disease estimates.

Patients at an early stage in the disease course, or asymptomatic or paucisymptomatic patients, might have low antibody concentrations that could give false-negative results. Patients' disease stage and severity are important points to consider, along with the population being tested. The estimated level of risk can be considered before using a serology test, because of the changing false-positive rate or low positive predictive value across different populations. Among the groups with the highest risk of the disease are symptomatic patients with clinical presentation of COVID-19, patients with other respiratory symptoms, contacts of confirmed cases, and health-care workers in settings with little personal protective equipment. We suggest countries consider risk levels before using serology tests and creating public health guidance. Scaling up testing, particularly at the community level, allows for better estimates of risks, which in turn allows more effective public health measures to be put into place than would be otherwise.

Testing to allow health-care workers to return to work

In a pandemic, countries must strive to maintain a robust health-care workforce. Key workers who develop

symptoms should be prioritised for molecular testing and receive care if infected. On recovery, should a serology test be used to decide when they can safely return to work? This strategy is based on the assumption that antibodies confer protective immunity.

Although antibodies against the receptor-binding domain of the spike protein and the nucleocapsid protein have been correlated with neutralising activity,[14,23,24] the development and duration of immunity has not yet been established.[34,35] Although it is tempting to speculate that serology tests based on the detection of neutralising antibodies can be used as markers of protective immunity, and people who test positive can get a so-called immunity passport to return to work, studies have shown that a significant proportion of patients remain RNA-positive despite high concentrations of antibodies against the receptor-binding domain of the spike protein and the nucleocapsid protein.[6,15,18,20,21] Wang and colleagues[36] found that elevated serum IgM concentrations are correlated with poor outcomes in patients with COVID-19 pneumonia, and Tan and colleagues[21] found that high concentrations of IgG antibodies were correlated with severe disease outcomes. Hence a substantial IgM or IgG response is not necessarily a surrogate marker of protective immunity. To date, insufficient evidence exists to recommend the use of serology testing for health-care workers to return to work. A negative molecular test remains the safest option to establish whether health-care workers can work again safely.

A policy brief by the World Bank suggested that serology testing could potentially have a high net benefit if it can allow dilution of restrictions for essential workers to return to work and revive essential segments of the economy.[37] The type of tests that can be used for immunity passports remains unclear. A better understanding of the interaction between infection and immune response dynamics is needed before these passports can be considered.

Testing to discharge patients from hospitals

Hospital beds are often in short supply. The recommended criteria for hospital discharge are two negative molecular tests over several days. However, molecular testing is often scarce or unavailable. Can serology tests be used for discharging recovered patients when molecular testing is not available?

As patients can remain positive for viral RNA despite rising concentrations of antibodies against the nucleocapsid protein and receptor-binding domain of the spike protein, which are correlated with neutralising activities, antibody tests cannot be used in the place of molecular tests to confirm that the patient is virus-free or at least no longer shedding live virus.

Conclusions

The events over the past few months have taught us that this pandemic is caused by an extraordinary pathogen that requires extraordinary measures to combat its spread and end the pandemic. The latest finding that as much as 44% of COVID-19 transmission happens before index cases become symptomatic[7] means that a great deal still needs to be learnt about this novel pathogen and its spread through a population. The paucity of knowledge on the dynamics of the immune response to infection has led to much hesitation on recommending the use of rapid immunodiagnostic tests, particularly serology tests.

On the basis of our current knowledge and understanding of viral infectivity and host response, we urge countries with restricted capacity for molecular testing to embark on research into the use of serology tests in triaging symptomatic patients in community settings, testing contacts of confirmed cases, and in situational analysis and surveillance. Rapid and scalable tests are needed to deal with this pandemic. Rapid serology tests, applied in the right situation for appropriate public health measures to be put into place, can make a huge difference. On Feb 10, 2020, leading health experts from around the world identified eight research and development priorities at the WHO R&D Blue Print meeting in Geneva, Switzerland, of which the top priority was to "mobilize research on rapid point of care diagnostics for use at the community level".[38] In line with this decision, research on the use of rapid serology tests to inform control programmes of their required performance and utility is an urgent priority in the COVID-19 pandemic response.

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