What is the role of testing in the COVID-19 pandemic?

Key Messages:

- SARS-CoV-2 testing plays an important role in the control of the COVID-19 pandemic. It enables diagnosis of cases to guide clinical management, facilitates identification of cases for isolation to reduce transmission, and provides estimates of prevalence at the population level to guide intervention implementation and resource planning.
- There are several molecular tests that detect the SARS-CoV-2 viral RNA in pharyngeal swabs (nasal or oral), and their use is dependent on the platforms available in the testing laboratories. To increase the testing capacity, all these platforms can be combined to maximize on the equipment and expertise available in different labs in the country while ensuring testing quality remains high across sites.
- Serological tests detecting either viral antigens in patient's blood or patient's antibodies against SARS-CoV-2 are also available for use. However, the accuracy of antigen and antibody detecting tests as clinical diagnostic tools have not been well established and require further studies.
- The appropriate application of these tests varies depending on the goal of testing and stage of disease. For the identification of active SARS-CoV-2 infection, RT-PCR tests are the current reference diagnostic standard while antibody detecting tests are appropriate for the identification of exposed individuals.
- Depending on the transmission pattern and testing capacity of a region, the population to be tested varies. In low transmission settings, testing all suspected symptomatic individuals meeting COVID-19 case definition and their close contacts is recommended. In high transmission settings with low testing capacity, targeted testing of priority groups (e.g. high-risk individuals, contacts of confirmed cases and healthcare workers) is suggested.

The role of testing in the control of COVID-19

Diagnostic testing to identify individuals infected with Severe Acute Respiratory Syndrome-coronavirus-2 (SARS–CoV-2) infection is crucial in the control of the COVID-19 pandemic. First, efficient and timely testing is a vital prerequisite for early identification and reporting of COVID-19. This, coupled with adequate contact tracing, isolation (of cases) and quarantine of contacts, is critical in preventing transmission and slowing down the spread of SARS-CoV-2. As a study in China recently reported, prior to the wide-scale movement restrictions in the country, undiagnosed SARS–CoV-2 represented the infection source for ~80% of reported cases[1]. Second, timely diagnosis facilitates early management of the disease to increase the recovery rate and lower mortality of COVID-19. Finally, testing provides accurate estimates of the presence and spread of SARS-CoV-2 in the population. Governments can use these estimates to inform resource planning and manage infection prevention and control interventions such as physical distancing while avoiding a major resurgence of transmission. For example, in South Africa, testing data has informed the development of a 5-tier risk adjusted strategy to ease lockdown restrictions based on incidence[2]. These observations emphasize the critical importance of wide-spread, accurate diagnostic testing in this pandemic. In the face of community transmission, the role of diagnostic testing is influenced by the type of testing available, the appropriate application of these tests and the population being tested.

Types of tests available

There are three types of tests developed for detection of SARS-CoV-2. Those that:

- detect viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR)
- detect viral proteins (antigen)
- detect specific IgM, IgG or IgA type antibodies produced in response to SARS-CoV-2

1.Viral RNA detection by RT-PCR (molecular tests)

These were the first tests to be developed and became the reference test for diagnosis[3]. Viral RNA can be detected from several clinical specimens such as the nasal and oropharyngeal swabs and

bronchoalveolar lavage (fluid from lung washings) using RT-PCR with high sensitivity and specificity[4,5]. Several RT-PCR assays have been developed and approved for use[6,7]. However, the accuracy of these tests relies heavily on the presence of the viral genome in sufficient amounts at the time and site of sample collection[8]. False negatives are more likely to occur early and later in the infection and with respiratory specimens obtained from the upper (nasal or oral swabs) versus lower respiratory tract (sputum or bronchoalveolar lavage)[6,8]. Similarly, an incorrect sample collection can limit the usefulness of the quantitative RT-PCR based assay[9]. RT-PCR testing usually takes 4 to 6 hours to complete, is complex and requires a high level of laboratory expertise. Because of this, RT-PCR testing is usually centralized in specialized laboratories. However, this slows down the identification of cases, as it requires special handling and shipping of clinical samples from different region to the laboratories. Alternatively, rapid, point-of-care (POC) molecular assays such as Cephid's Xpert Xpress, have been developed and have received emergency use approval (EUA) by the US Food and Drug Administration (FDA). Rapid POC tests are critical to expanding testing as time to result is less than an hour thus enabling quick isolation and timely clinical decisions after diagnosis. However, these tests still require a degree of expertise to set up and optimize in order to ensure high accuracy.

This type of test is appropriate for:

- Screening and confirmation of suspicious cases for isolation or treatment
- Detection of SARS-CoV-2 infection in contacts of confirmed symptomatic or asymptomatic cases
- Follow up on positive cases and to define when individuals can leave isolation facilities.

Antigen detection tests

These are tests that detect SARS-CoV-2 proteins[10]. Their applications would ideally be like those that detect viral RNA with the added benefit of fast time to results and low-cost for detection[10]. They utilize the lateral flow assay format which involves either a monoclonal antibody directed at a viral antigen or a viral antigen that is recognized by patients' antibodies immobilized onto a cassette. A positive result is visible as a colored line. Prototypes of such tests are under development[11] and over 20 have been granted emergency use approval by the FDA[12]. However, as specificity of these assays is vital to prevent false positives, a potential problem is the high similarity of coronavirus antigens[13]. In addition, these tests do not amplify the viral genome like the RT-PCR; therefore, when viral titers are low, sensitivity may be decreased. This limits their applicability in identifying active infections compared to RT-PCR. Although promising, the diagnostic accuracy of these tests in clinical settings is still under investigation.

Antibody detecting tests

Antibody detection tests, such as the enzyme-linked immunosorbent assays (ELISA), detect antibodies such as IgG and IgM to SARS-CoV-2 in clinical samples (e.g. blood, saliva or swab samples)[5,14,15]. These tests are less complex than RT-PCR, can provide results in 15-20 minutes and can be used for diagnosis in certain contexts such as late into disease when viral titers are lower[16]. However, the utility of these tests as a diagnostic tool is limited as antibody responses to infection takes days to weeks to be reliably detected[17-19]. Another potential problem is cross-reactivity with other coronaviruses; in which case a positive result may be due to past (or present) infection with other coronaviruses[16,18,20]. However, proper optimization of the tests can overcome this challenge. Antibody detection tests will be important for epidemiological studies i.e. serological surveys, vaccine studies and disease surveillance to understand how the population develops antibodies over the course of infection and how long these antibodies last. Commercial antibody detecting tests are already in the market in some countries. Testing sensitivity and specificity of these tests vary across available kits with sensitivity ranging from 77.1% for the Chembio Diagnostic Systems DPP Covid-19 IgM/IgG System to 100% in others[21]. In addition, local lab-based antibody assays that can be used for sero-surveillance are currently being developed. However, the accuracy of antibody tests as a diagnostic tool for SARS-CoV-2 is not well defined therefore these tests should not be used as the sole basis for diagnosis. However, they can be used in combination with RT-PCR tests.

This test is appropriate for:

- Serological surveys to estimate the percentage of population that is exposed and to make decisions about partial or definitive containment measures.
- A supplementary diagnosis tool in cases where molecular tests are negative but there is strong clinical suspicion of COVID-19.
- Selecting the population that can return to work

by identifying those with positive antibody tests. However, this is dependent on presence of sound evidence supporting the protective efficacy of antibodies against SARS-CoV-2.

- Seroprevalence studies to determine epidemiological variables of interest in public health, such as case fatality rate, attack rate and the expansion factor.

Use of test	Viral RNA detection by RT-PCR	Antigen detection	Antibody detection
Screening /during incubation period	Sufficient. However, viral titers may be too low for detection during incubation period. May yield false negative results very early in disease	Limited evidence but likely insufficient as it is dependent on viral titers. False negatives highly likely	Insufficient. False-negative highly likely early in disease
Identification of symptomatic cases	Current reference test	Limited evidence/unknown in clinical setting. Theoretically, able to detect although further studies on accuracy are needed	False-negative during early disease. May diagnose individuals presenting late with disease
Confirming viral clearance/de-isolation	Sufficient. However, low titers late in disease may lead to false negatives	Limited evidence. Likely insufficient. Sensitivity is low as viral titers decrease.	False-positive. Cannot distinguish between stages of disease
Epidemiological surveillance	Useful for passive surveillance	Limited evidence. Likely useful for passive surveillance	Serological surveys used to determine exposure and overall prevalence

Table 1: Selected use of the different types of SARS-CoV-2 diagnostic tests

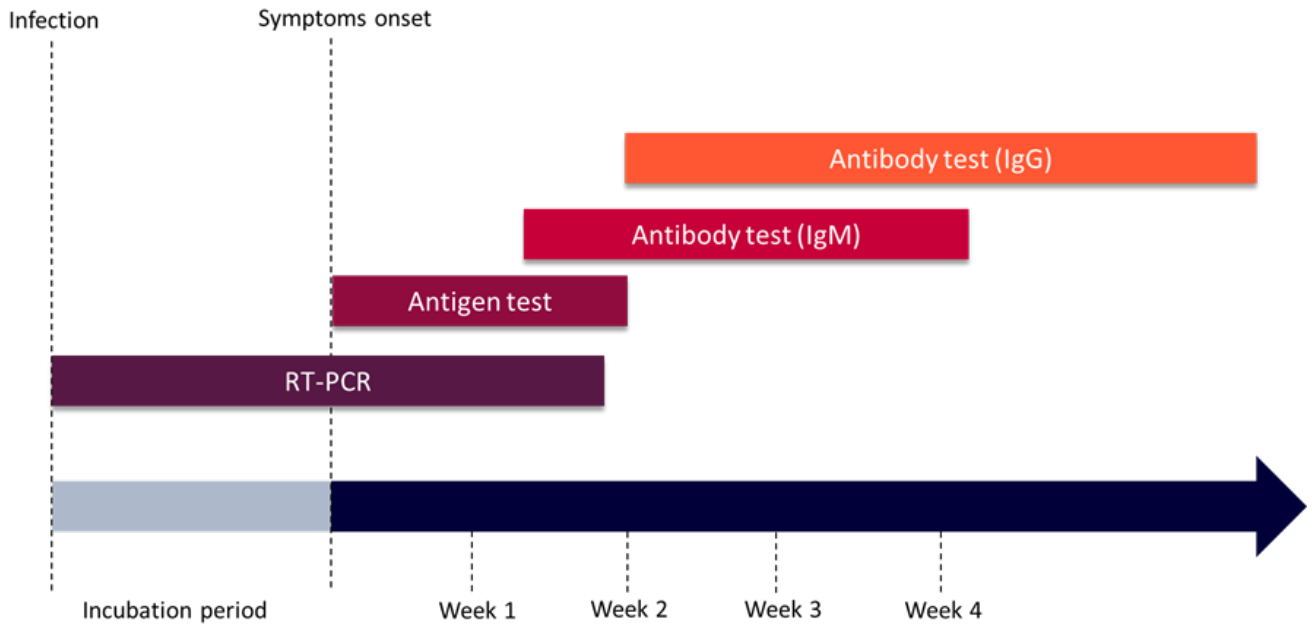

Figure 1: Testing windows after infection with SARS-COV-2[8,17-19].

SARS-CoV-2 tests used in Kenya

The Poisons and Pharmacy Board in Kenya has approved the use of four SARS-CoV-2 testing kits namely; The Xpert Xpress SARS-CoV-2 kit by Cephid, COBAS SARS-CoV-2 test by Roche Diagnostics, BioFire COVID-19 test by BioFire Defense and the Abbott RealTime SARS-CoV-2 assay by Abbott Molecular. A comparison of these tests is made in Table 2. Factors such as test performance, throughput, existing laboratory capacity,

the number and type of PCR platforms already available and cost are important to consider when choosing which RT-PCR tests to purchase. A limiting factor for these tests is that they exist on locked platforms and only kits designed by the manufacturers can be used. The development of testing protocols to be used on an open platform with several different kits is crucial. To increase the testing capacity, all these platforms can be combined to maximize on the equipment and expertise available in different labs in the country. However, to ensure the quality and consistency of testing remains the same in all laboratories, a coordinated effort that ensures assay optimization and protocols are standardized is required.

	Xpert Xpress SARS-CoV-2 test	COBAS SARS-CoV-2 assay	BioFire COVID-19 test	Abbott RealTime SARS-CoV-2 assay
Gene target	N2 and E-gene	ORF-1a/b and E-gene regions	ORF1ab and ORF8	RdRp and N gene
Limit of detection	0.01 pfu/mL	0.009 $TCID_{50}$/mL	3.3E+02 genomic copies/mL	100 viral copies/ml
Positive percent agreement/sensitivity	100%	100%	90%	95.2%
Negative percent agreement/specificity	100%	100%	100%	100%
Laboratory or point of care	Laboratory or at point of care	Moderate/high complexity laboratory	Moderate/high complexity laboratory	High complexity laboratory
Throughput	up to 2,000 samples in 24 hrs	The systems provide up to 96 results, a total of 384 results for the cobas® 6800 System and 1056 results for the cobas® 8800	264 samples per day	470 patient samples in 24 hours
Assay run time	30-45 minutes	3 hours for 96 samples	45minutes	7 hours
Specimen	Nasopharyngeal, nasal, mid-turbinate, or oropharyngeal swabs and nasal wash/aspirates	Nasopharyngeal and oropharyngeal swab	nasopharyngeal swab	Nasopharyngeal swabs, Oropharyngeal swabs
Authorization	FDA-EUA, CE-IVD	FDA-EUA, CE-IVD	FDA-EUA	FDA-EUA

Table 2: A comparison of the tests approved in Kenya for SARS-CoV-2 detection. The limit of detection (LoD) of a test represents the minimum amount of target that can be detected and quantified by the test.

Who should be tested?

In response to the rapidly evolving COVID-19 pandemic, countries have used different testing approaches depending on testing capacity, public health resources, and the spread of the virus in the community. In regions where there is no known circulation of SARS-CoV-2, sporadic cases and/or clusters of cases, all suspected individuals should be tested with emphasis on individuals with a travel history to high-risk areas[6].

In regions with community transmission of SARS-CoV-2, mass testing is useful. This involves testing even people who have no symptoms. In the context of SARS-CoV-2, this approach is important based on several observations. One, asymptomatic individuals may be a substantial source of transmission. Some studies have assessed the proportion of asymptomatic individuals and report proportions ranging from

5%-85%[22-26]. Second, transmission can occur before onset of illness[27-30]. Third, this approach has been effective in some regions. The town of Vo'Euganeo in Italy managed to reduce its number of cases by 90% by repeat testing its entire population and isolating the infected[23]. In South Korea and Germany, success in containing the virus has been credited to mass testing coupled with aggressive contact tracing and isolation[2]. Iceland has so far tested 12% of its population and have managed to contain the spread of the virus[31]. However, mass testing is extremely expensive and logistically challenging. Furthermore, testing capacity in most countries would not be enough for population-wide testing. Therefore, in countries with community spread of SARS-CoV-2 and limited testing capacity, testing must be prioritized/targeted. This allows governments to maximize test availability for critical populations. The WHO and CDC have outlined testing prioritization recommendations[6,32]. Priority should be given to frontline health workers, individuals who are at risk of developing severe disease, hospitalized patients with respiratory symptoms and the first symptomatic individuals in a closed setting (e.g. hospitals, prisons and care homes). Once testing capacity has been increased, testing can be expanded to suspected mild cases and contacts of confirmed cases. A second priority group that can be considered for testing are individuals who come into contact with many other people as part of their daily activities such as public transport and supermarket workers, police and other essential public workers. These groups are not only at higher risk of exposure but can also infect many people. Targeted testing can also focus on geographical clusters and regions with sporadic outbreaks to determine how stringent restriction measures can be[6].

Scenario	Who to test	Laboratory confirmation
No known SARS-CoV-2 circulation	• All suspected cases. • Intensified testing can target individuals with recent travel to high-risk countries	• A positive RT-PCR result for at least two different targets on the COVID-19 virus genome.
Sporadic cases	• All suspected cases. • Each sporadic case requires aggressive and active case finding, isolation and care, and comprehensive contact tracing and quarantine.	• A positive RT-PCR result for at least two different targets on the COVID-19 virus genome.
Cluster of cases	• All suspected cases. • Intensify investigation of cases and clusters and SARI/ILI surveillance. • Plans should be adopted to improve national testing capacity.	• Screening by RT-PCR of a single discriminatory target.
Wide-spread/community transmission	• All suspected cases. • Where capacity does not meet needs, testing priority should be given to vulnerable patients and health care workers • Plan to significantly increase the number of individuals that need to be tested for COVID-19.	• Screening by RT-PCR of a single discriminatory target is considered sufficient. • In cases where RT-PCR is negative but there is strong clinical suspicion of COVID-19, serological tests can be used.
Seroepidemiological screening to Identify all exposed to infection, defining attack rates, case fatality rates, and infection fatality rates	• The geographic scope of the investigation must be defined. i.e. local, regional or national. Population tested should be representative of the overall burden of infection (i.e. include both high and low incidence areas) and over a range of ages. Sampling can be random or convenient (e.g. blood donors) • Suspected or confirmed COVID-19 patients should not be excluded!	• Total antibodies or IgG should be detected using enzyme linked immunosorbent assay (ELISA), immunofluorescence (IFA) or, in case of limited lab capacity, Rapid Diagnostic Tests (RDT)

Table 3:SARS-CoV-2 testing guidance based on transmission patterns and epidemiological investigation6,32

Conclusion

SARS CoV-2 testing is critical for informing decisions for the management and control of the pandemic. The viral RNA tests (RT-PCR) and the antigen and antibody detection tests have different scopes and their use and interpretation should be adjusted to the clinical or epidemiological context. However, to ensure testing quality, these tests should be optimized and standardized across all testing sites.

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This brief was prepared by: Fatuma Guleid, Dr. Sam Akech, Dr. Isabella Oyier and Dr. Edwine Barasa

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