verily Covid-19 Testing

Multiple molecular methods are available for COVID-19 testing. Here are some of the pros and cons of each.

Introduction

At the end of July 2020, the number of global COVID-19 cases reached 17.7 million and 681,341 deaths. In the U.S. alone, more than 4.6 million individuals had tested positive for the virus that causes COVID-19, and more than 153,000 had died [1]. The COVID-19 pandemic has generated a dramatic and rapid scientific response and has prompted the development of diagnostic technologies at an unprecedented pace. Here, we provide a brief overview of the current testing landscape and a summary of decisions that Verily's COVID-19 testing team has implemented for research and testing programs.

Major Types of COVID-19 Testing

In general, there are two different types of tests: **molecular tests** and **serology/antibody tests** (See **Table 1**). **Molecular tests** detect parts of the SARS-CoV-2 virus, such as the virus's genetic material or specific proteins on the surface of the virus. A molecular diagnostic test can show if an individual has an active coronavirus infection and if they should take steps to quarantine or isolate themselves from others. **Antibody tests**, also known as serology or serological tests, are blood-based tests that measure the presence of antibodies to the SARS-CoV-2 virus. As such, antibody tests can serve as a historical record of a previous exposure or infection, but are not as useful for detecting active infections.

Molecular Tests

Molecular tests are recommended to diagnose an active infection. Authorized molecular tests include those that detect SARS-CoV-2 nucleic acids (**nucleic acid tests)**, or those that detect surface antigens (**antigen tests**). Acceptable sample types for diagnostic tests now include nasopharyngeal, oropharyngeal, mid-turbinate, or anterior nares swabs, as well as nasopharyngeal or nasal wash/aspirate specimens. Lower respiratory tract specimens, such as sputum, bronchoalveolar lavage, tracheal aspirate, pleural fluid and lung biopsy are only collected under specific clinical circumstances [2]. Recently, the FDA granted Emergency Use Authorization (EUA) to a saliva-based test that allows a patient to spit into a tube, thereby facilitating remote self-collection [3].

Although antigen tests can offer fast and scalable test performance, they often exhibit lower sensitivity than most nucleic acid-based assays. Therefore, the majority of diagnostic methods for COVID-19 testing are still nucleic acid-based assays, targeting one or more of the nucleocapsid (N), RNA-dependent RNA polymerase (RdRP), envelope (E), spike protein (S), and/or ORF1ab encoding regions of the SARS-CoV-2 genome (**Table 1**). Nucleic acid-based assays are highly sensitive and specific when conducted in centralized laboratories with standardized protocols,

but often require large lab spaces, complex equipment, regulatory approvals, and skilled technicians [4]. For certain use cases, low-complexity molecular diagnostic point-of-care tests with rapid turnaround can have substantial practical advantages, and a number of point-of-care diagnostic tests have now received EUA by the FDA [5].

A negative PCR test does not rule out COVID-19 infection. The sensitivity of SARS-CoV-2 molecular tests can vary greatly depending on the collection timing relative to the initial infection, collection methodology, and physiological location sampled. Also, PCR tests will not indicate if a person was infected at some point in the past and has subsequently recovered.

Antibody Tests

Antibody tests, or serology tests, require whole blood, plasma, or serum samples from patients and can detect different types of antibodies, such as IgG, IgM, and IgA. These antibodies are generated by the body's immune system and target proteins of the SARS-CoV-2 virus. Detection of these antibodies can be achieved through well-established methods, including enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), or lateral flow assays (**Table 1**). Other methods, like neutralization assays, can identify if a patient has antibodies in the serum that are active and effective against the virus by blocking its ability to infect cells in a lab-based cell culture environment. However, the necessary levels of neutralizing antibodies required for protection are still unknown, and no reliable neutralization tests are currently authorized [6].

It can take several days or weeks to develop enough antibodies to be detected in a test after viral infection. For this reason, the CDC does not currently recommend using antibody testing as the sole basis for diagnosis of acute infection, and antibody tests are not authorized by FDA for diagnosis of infection [7]. It is also important to note that antibody test performance thus far has exhibited a broad range of variability in performance; therefore, the CDC advises choosing an FDA-authorized antibody test with high specificity and only testing people who are likely to have had COVID-19 in order to minimize false positives [6, 8].

Although antibody tests cannot be used to diagnose whether someone currently has an active infection, they can help us understand the transmission dynamics of the virus in the general population and provide information about populations that may be immune.

	Molecular tests		Antibody tests
	Nucleic acid tests	Antigen tests	
Sample collection	Nasal or throat swab Saliva	Nasal or throat swab	Blood draw
Turnaround time	<1 - 4 hours	<1 hours	<4 hours
Methods	RT-PCR RT-LAMP ddPCR CRISPR Sequencing	Lateral Flow	ELISA CLIA ECLIA FMIA CMIA Lateral Flow
Source	Viral RNA	Viral antigen	IgG, IgM
Target	E, S, N, ORF1ab	E, S, N	S, N
Authorized settings	Lab-based Point of care	Lab-based Point of care	Lab-based

Abbreviations: ELISA = enzyme-linked immunosorbent assay; CLIA = chemiluminescence immunoassay; ECLIA = electrochemiluminescence immunoassay; FMIA = fluorescent microsphere Immunoassay, CMIA = chemiluminescent microparticle immunoassay.

Table 1. Types of molecular and antibody tests [9, 10]

Diagnostic	Limit of Detection (genome copies/mL)	Inclusivity (number of SARS CoV-2 references)	Cross-Reactivity (number of organisms tested)
BD Max BioGX SARS-CoV-2	40	N1: 98% N2: 99.5% (3634 GISAID)	not stated (CDC)
Euroimm EUORealTime SARS-CoV-2	150	N1: 100% ORF1ab: 100% (1534/1523 GenBank)	no cross-reactivity (28 *in vitro*) ≥80% homology for two primers (26 *in silico*)
Fulgent COVID-19 by RT-PCR	5000	N1: 99.2% N2: 99.8% (1298 GenBank)	100% homology for one primer (77,943 *in silico*)
LabCorp COVID-19 RT-PCR	6250	not stated	SARS-CoV (26 *in vitro*) no homology (41 *in silico*)
Roche cobas SARS-CoV-2	25	T1: 99.8% T2: 99.3% (445/445 NCBI/GISAID)	SARS-CoV (41 *in silico*)
Thermo TaqPath COVID-19	250	99.99% (25998 GenBank /GISAID)	≥80% homology for one primer (43 *in silico*)
Verily COVID-19 RT-PCR	60	99.99% (25998 GenBank /GISAID)	≥80% homology for one primer (43 *in silico*)
Verily 2D Pooling COVID-19	720	99.99% (25998 GenBank /GISAID)	≥80% homology for one primer (43 *in silico*)

Table 2. Performance of a selection of nucleic acid diagnostic tests [5]. The limit of detection is the lowest concentration of analyte in a specimen that can be consistently detected ≥95% of the time. Inclusivity refers to the fraction of SARS-CoV-2 genomes on which the diagnostic's primers and probes are predicted to map and produce signal. The percent homology of each assay component is listed with the number of reference SARS-CoV-2 sequences tested in silico. Here, the TaqPath analysis deemed mapping successful for a given isolate if at least two of the three targets showed 100% identity. Cross-reactivity refers to the predicted or measured activity for a diagnostic's primers and probes for pathogens other than SARS-CoV-2. Reported information includes: the homology of any high scoring assay components, results from in vitro tests, and the number of organisms tested (in vitro, in silico, or both). Verily inclusivity and cross-reactivity is assumed equivalent to TaqPath, the test it is based on.

Diagnostic Test Parameters to Consider

Taken together, there are a number of important parameters to evaluate when making decisions around what testing options to establish in a clinical laboratory and/or testing program. This includes the performance of the assay, the cost per test (including reagents, consumables, capital equipment, and technician time), the scalability of the workflow, the end-to-end run time, and the robustness of the supply chain.

Table 2 illustrates the reported performance, based on the limit of detection, of a selection of diagnostic tests with EUA. Note that the matrix used for the limit of detection determination can directly influence the test performance, and so direct comparisons may require some caution in interpretation. There is also a substantial amount of heterogeneity in how different diagnostics assess inclusivity and cross-reactivity, both in the number of

in silico reference sequences evaluated, and whether or not any *in vitro* tests were conducted.

Based on the above criteria, Verily adopted the ThermoFisher TaqPath COVID-19 Combo Kit EUA test as a core nucleic acid based diagnostic. This test was selected based on the high performance of the assay, the reliability of an established manufacturer to supply reagents at scale, and ThermoFisher's early EUA. In addition, by implementing minor changes to the authorized TaqPath workflow, Verily developed a more sensitive version of the original assay. In addition, Verily has developed a sample pooling-based workflow to further increase throughput, conserve reagents, reduce costs. Refer to Verily's white paper "Verily's Approach to COVID-19 Diagnostic Testing" for additional details.

For serology testing, Verily has established the Roche Elecsys Anti-SARS-CoV-2 assay as a core immunoassay for IgG and IgM antibody detection in human blood, serum, and plasma. This test was selected based on performance (100% sensitivity, 99.8% specificity, 96.5% positive predictive value at 5% prevalence, and 100% negative predictive value at 5% prevalence), the reliability of the manufacturer to supply reagents at scale, and the simplicity of operation.

National Testing Efforts

To meet the demanding diagnostic challenges that have emerged during this pandemic, Verily launched an initiative to ramp up COVID-19 testing availability. On March 15, 2020, in partnership with the California Governor's office, local county governments, and public health authorities, Verily launched the Baseline COVID-19 Testing Program to expand the availability of COVID-19 screening and testing, beginning in California [11]. By June 15, 2020, the Baseline COVID-19 Testing Program has been expanded to 130 locations nationwide, providing over 256,844 tests [12], and by the end of July, to 300+ locations nationwide, providing over half a million tests. In parallel, Verily launched a Baseline COVID-19 Research Project to advance scientific understanding of the disease and for developing treatments [13, 14]. By August 13, 2020, more than 50,000 members across 50 states joined the COVID-19 Research Project. On June 18, 2020, Verily launched the Healthy at Work Program to assist organizations in their efforts to ensure a safe return to work and school [15].

At Verily, we are leveraging our expertise in user engagement platforms, scalable testing sites, high throughput laboratory workflows, and data analytics to better understand COVID-19 and help achieve better community health outcomes.

References

1. Johns Hopkins University & Medicine, Coronavirus Resource Center Data
2. CDC: Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (Updated July 8, 2020)
3. FDA: Coronavirus (COVID-19) Update: Daily Roundup May 8, 2020
4. COVID-19 Diagnostics In Context (Updated 08/02/2020)
5. FDA: In Vitro Diagnostics EUAs (Updated July 31, 20)
6. CDC: Interim Guidelines for COVID-19 Antibody Testing (Updated Aug. 1, 2020)
7. CDC: Overview of Testing for SARS-CoV-2 (Updated July 17, 20)
8. FDA: EUA Authorized Serology Test Performance (Updated July. 16, 2020)
9. The COVID-19 Diagnostic Technology Landscape: Efficient Data Sharing Drives Diagnostic Development. *Front. Public Health*, 18 June 2020
10. FDA: Coronavirus Testing Basics (Updated July 16, 2020)
11. Verily: The Project Baseline COVID-19 Program: Responding to a health crisis
12. Verily: Verily Expands Baseline COVID-19 Testing Program to 130 Locations Nationwide, Providing Over 256,844 Tests

13. Verily: New Baseline COVID-19 Research Project launches (May 18, 2020)
14. Verily: A Look At The COVID-19 Research Community (July 9, 2020)
15. Verily: Verily Launches Healthy at Work Program to Support Organizations In Their Efforts to Ensure a Safe Return to Work and School

Supplemental Information

Several platforms for open and expedient data sharing have contributed to the development and application of emerging diagnostic tests. Up-to-date information on COVID-19 diagnostic technology can be found at the following sources:

- WHO Coronavirus disease (COVID-19) technical guidance
- U.S. FDA Coronavirus Disease 2019 (COVID-19)
- Johns Hopkins University & Medicine, Coronavirus Resource Center Data
- FIND: COVID-19 Diagnostics Resource Center
- GISAID genomic data of hCoV-19
- COVID-19 Testing Project
- medRxiv: preprint servers for early publication of research studies
- bioRxiv: preprint servers for early publication of research studies