Editors' Note:

The co-editors of the Hawai'i Journal of Health & Social Welfare extend our warmest wishes to our readers during the COVID-19 pandemic. Our health care brethren around the world and here in Hawai'i are bringing their collective expertise to help quell the pandemic and minimize the loss of human life. In this issue, we are pleased to offer three non-peer reviewed guest columns to help our readers understand some of the key issues of COVID-19. Stay safe and be well. — ***S. Kalani Brady and Tonya Lowery St. John***

COVID-19 Special Column: Principles Behind the Technology for Detecting SARS-CoV-2, the Cause of COVID-19

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Abstract

Nationwide shortages of tests that detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and diagnose coronavirus disease 2019 (COVID-19) have led the US Food and Drug Administration (FDA) to significantly relax regulations regarding COVID-19 diagnostic testing. To date the FDA has given emergency use authorization (EUA) to 48 COVID-19 in vitro diagnostic tests and 21 high complexity molecular-based laboratory developed tests, as well as implemented policies that give broad authority to clinical laboratories and commercial manufacturers in the development, distribution, and use of COVID-19 diagnostic tests. Currently, there are 2 types of diagnostic tests available for the detection of SARS-CoV-2: (1) molecular and (2) serological tests. Molecular detection of nucleic acid (RNA or DNA) sequences relating to the suspected pathogen is indicative of an active infection with the suspected pathogen. Serological tests detect antibodies against the suspected pathogen, which are produced by an individual's immune system. A positive serological test result indicates recent exposure to the suspected pathogen but cannot be used to determine if the individual is actively infected with the pathogen or immune to reinfection. In this article, the SARS-CoV-2 diagnostic tests currently approved by the FDA under EUA are reviewed, and other diagnostic tests that researchers are developing to detect SARS-CoV-2 infection are discussed.

Keywords

COVID-19, SARS-CoV-2, RT-PCR, molecular diagnostic testing, serological diagnostic testing

Abbreviations

ACE2 = angiotensin-converting enzyme 2
cDNA = complementary DNA
CDC = Centers for Disease Control and Prevention
CLIA = clinical laboratory improvement amendments
COVID-19 = coronavirus disease 2019
DNA = deoxyribonucleic acid
E = envelope protein
ELISA = enzyme-linked immunosorbent assay
EUA = emergency use authorization
FDA = Food and Drug Administration
IgA = immunoglobulin A
IgG = immunoglobulin G
IgM = immunoglobulin M
LFIA = lateral flow immunoassay
M = membrane protein
MERS-CoV = Middle East respiratory syndrome coronavirus
MIA = microsphere immunoassay
N = nucleocapsid protein
NAT = nucleic acid test
NAAT = nucleic acid amplification test
nsp = non-structural protein
ORF = open reading frame
POC = point-of-care
qRT-PCR = real-time reverse transcription-polymerase chain reaction
RNA = ribonucleic acid
RP = human RNase P protein
S = spike protein
SARS-CoV = severe acute respiratory syndrome coronavirus
SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2
WHO = World Health Organization

Introduction

In late 2019 an outbreak of pneumonia of unknown etiology emerged in Wuhan City, Hubei Province, China, and quickly spread throughout the world.[1] On March 11, 2020, the WHO declared the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causative agent of coronavirus disease 2019 (COVID-19), a global pandemic, as the numbers of cases outside of China began to eclipse those found within the country.[2] Since then, cases of COVID-19 have been reported in more than 200 countries, areas or territories worldwide.[3] Recent reports of the outbreak in China, have demonstrated the important role of mild to asymptomatic SARS-CoV-2 infections in viral transmission, estimating that as many as 86% of infections were undocumented with mild, limited, or no symptoms.[4] Therefore, access to accurate and timely testing and detection of the virus is essential to limiting the spread of SARS-CoV-2.

The Centers for Disease Control and Prevention (CDC) developed the first diagnostic test approved for clinical detection of SARS-CoV-2 and diagnosis of COVID-19 in the United States (US). The CDC COVID-19 diagnostic panel is a real-time

reverse transcription-polymerase chain reaction (qRT-PCR) test. In qRT-PCR, oligonucleotide primers are used to amplify pieces of nucleic acid (ie, RNA or DNA), which can be detected by a fluorescently labeled probe. In the CDC diagnostic test, 2 regions of the SARS-CoV-2 nucleocapsid (N) gene, as well as an internal control, the human RNase P gene (RP), are amplified. Detection of all 3 genes is considered presumptive positive for SARS-CoV-2, in conjunction with a patient's clinical signs/symptoms and/or epidemiological criteria for COVID-19 infection (ie, travel history, close contact with a confirmed COVID-19 case).[5]

Early technical issues with this CDC-developed COVID-19 diagnostic panel, coupled with logistical and technical difficulties in large-scale manufacturing of diagnostic tests for a rapidly emerging COVID-19 disease, has led to widespread shortages of diagnostic tests throughout the US. To address these shortages, the Food and Drug Administration (FDA) has given emergency use authorization (EUA) for 41 molecular diagnostic tests (Table 1 [http://hawaiijournalhealth.org/past_issues/COVID-19_Diagnostics_Table1.xlsx]), 21 high complexity molecular-based laboratory developed tests (Table 2 [http://hawaiijournalhealth.org/past_issues/COVID-19_Diagnostics_Table2.xlsx]), and 7 serological diagnostic tests (Table 3 [http://hawaiijournalhealth.org/past_issues/COVID-19_Diagnostics_Table3.xlsx]) to date.[6] EUA is a mechanism by which the FDA fast tracks diagnostic and therapeutic medical devices to diagnose and respond to public health emergencies such as COVID-19. EUA devices are not FDA licensed, however, an EUA application has been reviewed and approved by the FDA for these devices. These EUA in vitro diagnostic tests include molecular diagnostics (that detect viral RNA sequences) and serological tests (that detect antibodies [ie, IgA, IgG, IgM] directed towards viral antigens). Furthermore, on March 16, 2020, the FDA released a COVID-19 diagnostic guidance document that enacted several unprecedented policy changes for diagnostic procedures during a public health emergency.[7] Briefly, the FDA enacted 4 new policies regarding COVID-19 diagnosis that: (A) Allow clinical laboratory improvement amendments (CLIA) certified laboratories capable of high-complexity testing to use internally validated tests prior to EUA submission; (B) expand state authority over requirements for high-complexity testing; (C) allow commercial manufacturers to develop and distribute tests prior to EUA submission; and (D) allow commercial manufacturers to develop and distribute serology tests without an EUA. These policies gave sweeping authority to CLIA-certified laboratories and commercial manufacturers to use COVID-19 diagnostic tests in a clinical setting without FDA review.

Basic Virology of SARS-CoV-2

SARS-CoV-2 belongs to the *Coronaviridae*, a family of large, enveloped, positive-sense, single-stranded RNA viruses known to infect a wide variety of animals. Prior to 2003, these viruses were thought to cause only mild, common cold-like disease in humans. SARS-CoV-2 is the seventh coronavirus known to infect humans, including the 4 common cold coronaviruses (229E, OC43, NL63, and HKU1) and 2 other strains, known to cause severe pneumonia associated respiratory disease that can become fatal: severe acute respiratory syndrome coronavirus (SARS-CoV), which emerged in 2003, and Middle East respiratory syndrome coronavirus (MERS-CoV), which emerged in 2012.[8] SARS-CoV-2 is also known to cause a severe pneumonia associated respiratory disease, which can become fatal in humans. SARS-CoV, MERS-CoV, and SARS-CoV-2 all have zoonotic origins, emerging in human populations from spillover events, which occur when a pathogen-carrying animal reservoir comes into contact with a novel host population, in this case humans. Genomic sequencing has demonstrated that SARS-CoV-2 is closely related to 2 bat-derived SARS-like *Betacoronaviruses*.[9]

SARS-CoV-2 Genome

The genome of SARS-CoV-2 is approximately 30-kb in length and consists of 6 open reading frames (ORFs), which includes ORF1a/b, spanning 16 non-structural proteins (nsp) relating to the replication-transcription complex, 4 structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N), along with several other non-structural, special structural, and/or accessory ORFs (ORF3a/b, 6, 7a, 7b, 8, and 10).[9-11] Genome sequencing alignment among different coronaviruses reveals more conservation among the non-structural proteins (58% identity) as compared to structural proteins (48% identity).[12] The diversity in structural proteins has allowed coronaviruses to adapt to new hosts. Therefore, sensitive and specific diagnostic techniques should target both structural and non-structural proteins. Multiple sequence alignment and phylogenetic analysis of SARS-CoV-2 among 95 strains of the virus isolated from COVID-19 patients around the world have revealed high sequence homology at both the nucleotide level (99.99%; range 99.91-100%) and the amino acid level (99.99%; range 99.99-100%).[10, 13] This indicates a low mutation rate for SARS-CoV-2.

SARS-CoV-2 Diagnostic Targets

Most diagnostic tests target a combination of structural (S, N, and/ or E) and non-structural (ORF1ab region) SARS-CoV-2 genes, along with positive and negative controls. This testing strategy ensures that the diagnostic targets include a non-structural protein, highly conserved across coronaviruses, as well as structural protein(s), highly specific for SARS-CoV-2.

S protein. Much of the literature has focused on understanding the mechanisms by which the clubbed-shaped SARS-CoV-2 S protein allows the virus to enter host cells. The S protein of SARS-CoV-2 binds to the host cells and enters the cell using the angiotensin-converting enzyme 2 (ACE2) cell receptor.[9, 14] SARS-CoV S protein also utilizes the ACE2 cell receptor to enter host cells; however, genetic analysis has demonstrated

that the 2 viruses bind to the receptor at different amino acid residues. Further, SARS-CoV-2 binding is stronger than that of SARS-CoV, suggesting that this new virus is more successful at viral entry, providing a mechanism to explain the explosive spread of SARS-CoV-2 as compared to SARS-CoV.[15] Wrapp and colleagues reported that the SARS-CoV-2 spike protein's binding affinity to ACE2 is 10-20 times higher than that of the SARS-CoV spike protein, suggesting efficient person-to-person transmission.[16] Among the structural proteins, the highest sequence diversity between SARS-CoV and SARS-CoV-2 occurs in the S protein (24%).[10,13] Therefore, use of the spike protein as part of SARS-CoV-2 diagnostic testing will provide high specificity.

N protein. The N gene encodes a ribonucleoprotein that contains the viral genome.[8] There is little sequence diversity in the N gene between SARS-CoV and SARS-CoV-2 (9.6%).[13] Further, the mechanism by which coronaviruses replicate and transcribe their genomes results in a set of nested RNAs.[11,17] As the N gene sits at the end of the coronavirus genome, this nested strategy makes the N gene the most abundant nucleotide sequence during virus replication, and therefore an excellent diagnostic target.[18] Furthermore, because SARS-CoV is currently rare in human populations, this conserved region remains a useful target for SARS-CoV-2 detection.

E protein. The E gene encodes a small polyprotein found in low amounts in all coronavirus envelopes.[8] There is little sequence diversity in the E gene between SARS-CoV and SARS-CoV-2 (5.3%).[13] A recent report by Corman and colleagues has demonstrated high specificity for the E gene in COVID-19 diagnostic testing.[19] During viral replication the E gene is present in such low abundance that replication of these sequences has high positive predictive value for infection with SARS-CoV-2.

ORF1ab segment. ORF1ab comprises two-thirds of the SARS-CoV-2 genome, encoding 16 nsp relating to the replication-transcription complex responsible for all the machinery associated with viral replication, such as the RNA-dependent RNA polymerase (nsp12; RdRp), and a 3'-5' exoribonuclease (nsp14; ExoN).[8] ExoN is a unique feature of the replication-transcription complex of all coronaviruses, conferring a unique proofreading mechanism across the virus family and resulting in a low mutation rate among coronaviruses. The essential role of the replication-transcription complex, in particular RdRp, in viral replication, makes targeting regions of ORF1ab desirable for diagnostic tests.[11]

Strategy for Testing SARS-CoV-2 Infection

Symptomatic Infection

The primary symptoms of COVID-19 include fever, cough, and shortness of breath.[20,21] There is growing evidence of gastrointestinal symptoms (ie, abdominal pain, diarrhea, nausea, vomiting), as well as altered sense of smell and taste from US and international case reports.[22] With severe diagnostic testing shortages, the CDC has developed guidance for clinicians on testing people suspected to have COVID-19. This guidance ranks different groups in decreasing level of testing priority: (1) hospitalized patients and healthcare facility workers with symptoms; (2) high risk individuals (ie, individuals in long-term care facilities, >65 years, with underlying conditions) and first responders with symptoms; (3) critical infrastructure workers and any other individuals with symptoms, healthcare facility workers and first responders without symptoms, and individuals with mild symptoms in communities experiencing high numbers of COVID-19 hospitalizations.[23]

Asymptomatic Infection

Person-to-person spread via respiratory droplets is thought to be the primary route of transmission for SARS-CoV-2. Direct transmission via fomites, the fecal-oral route, and aerosol particles have been speculated as other important routes of transmission.[24] The role of asymptomatic and even pre-symptomatic individuals has slowly emerged as important sources of transmission in the ongoing pandemic.[4] Asymptomatic refers to individuals who become infected and shed infectious particles without developing symptoms, while pre-symptomatic refers to the shedding of infectious particles prior to symptom onset. Virus transmission by these populations can be very successful, as these individuals do not know that they are carrying the virus, and consequently may not be taking any of the recommended precautions (social distancing, hand-washing, etc.). Due to diagnostic shortages, the CDC considers potentially exposed individuals with no symptoms of COVID-19 to be a low priority for testing.

COVID-19 Situation in Hawai'i

The first confirmed case of COVID-19 in the US was reported on January 20, 2020 in a 35-year old male in Snohomish County, Washington, who had history of travel to Wuhan, China.[21] The first confirmed case of COVID-19 in a resident of Hawai'i was reported on March 6, 2020, in a person who had returned home from a cruise.[25] As of April 25, 2020, there have been 604 confirmed cases of COVID-19 in the state of Hawai'i, of which 11% (n=68) have required hospitalization. Fourteen people have died of the infection (2.3% case fatality rate [CFR]). Confirmed COVID-19 cases have been reported in all 4 Hawai'i counties, however Honolulu County accounts for 65% (n=395) of the state's cases.[26] More than 80% of the cases have been associated with travel or travel-associated contact.[26] Similar to the nationwide response, the state of Hawai'i has been under a stay-at-home, work-at-home order since March 25, 2020, with exemptions for essential workers.[27] Further, 14-day self-quarantine for all visitors and residents returning to Hawai'i was implemented on March 26, 2020,[28] and was extended to include interisland travelers on April 1, 2020.[29] All

these restrictions are been accompanied with fines and possible imprisonment for individuals who do not follow these quarantine requirements and/or social distancing measures.

Diagnostic testing for COVID-19 is being conducted by the Hawai'i State Laboratories Division, commercial laboratories (ie, Clinical Labs of Hawaii, Diagnostic Laboratory Services), as well as hospital laboratories (ie, Kaiser Permanente). The majority of the tests are sent to commercial laboratories on the US mainland, resulting in extended delays for individuals waiting for the results of their COVID-19 testing.[30] As of April 25, 2020, there have been 27,572 tests performed by commercial clinical and state laboratories for the state of Hawai'i, with a 2.2% rate of COVID-19 positivity.[31] The state has adopted CDC's COVID-19 testing guidance to determine testing priorities, expanding these priorities to include asymptomatic close-contacts of confirmed COVID-19 cases.

Molecular Diagnostic Tests

Sample Collection and Preparation for Molecular Testing of COVID-19 and RNA Extraction

Typically, specimens for detecting SARS-CoV-2 are collected from upper or lower respiratory tracts of individuals with clinical signs/symptoms and/or who fulfill the epidemiological criteria for COVID-19. Upper respiratory tract specimens include nasopharyngeal, oropharyngeal swabs, and nasal wash/aspirates. Collection of such specimens is quick and easy, without requirement of specialized skills and/or equipment. Lower respiratory tract specimens include sputum, tracheal aspirates, and bronchoalveolar lavage fluid. These specimens require much more invasive techniques and are typically collected from hospitalized patients. In patients with active SARS-CoV-2 infections, these specimens contain cells with SARS-CoV-2 viral RNA. Samples can be stored at 2-8ºC for 72 hours, or -70ºC or below if delays in testing are anticipated. Following specimen collection, the cells are lysed and total RNA is isolated from the cells. Total RNA can be isolated through a variety of techniques, such as using a spin column or magnetic beads. The procedures include RNA binding steps and sample washing to remove all other cellular products (ie, DNA, proteins, organelles).[23]

Tests and Their Principles Employed for Detection of COVID-19

A nucleic acid test (NAT) is used in infectious disease diagnostic testing to directly detect nucleic acid (RNA or DNA) sequences of the suspected pathogen. From patient specimens, total nucleic acid is extracted, including nucleic acid from the host; therefore, the amount of genetic material of the suspected pathogen in the sample can be very low, making direct detection of the pathogen's nucleic acids difficult. As a result, the genetic material of interest must be amplified by creating millions of copies of sequences specific to the pathogen of interest, for easier detection. This is why this is called a nucleic acid amplification test (NAAT). For most current diagnostic tests available for COVID-19, amplification of RNA specific for the SARS-CoV-2 virus is accomplished using qRT-PCR.

Real-time reverse transcription polymerase chain reaction (qRT-PCR). Most of the COVID-19 diagnostics with FDA EUA use the technique of qRT-PCR, in which a specific sequence(s) of the SARS-CoV-2 genome is amplified and detected with fluorescently labeled probe(s), quantifying patient's viral copy number in real-time. Detection by qRT-PCR is the most sensitive and specific diagnostic tool currently available.

By this technique, following RNA isolation from the specimen, the unstable single-stranded RNA must be stabilized into double stranded molecules by adding complementary DNA (cDNA) by reverse transcription. The isolated RNA with cDNA can then be amplified using oligonucleotide primers and fluorescently labeled probe(s) specific to region(s) of the SARS-CoV-2 genome by PCR. At this time, 39 qRT-PCR diagnostic assays have been given FDA EUA, of which 6 are only approved for use in specific commercial CLIA laboratories (Wadsworth Center, New York State Department of Health (New York, NY); Laboratory Corporation of America (Burlington, NC); Quest Diagnostics Infectious Disease, Inc. (San Juan Capistrano, CA); Avellino Lab USA, Inc. (Menlo Park, CA); Ipsum Diagnostics, LLC (Atlanta, GA); and KorvaLabs, Inc. (Menlo Park, CA)) (Table 1). Further, the FDA has given EUA to an additional 18 high complexity molecular-based laboratory developed tests that are approved for use only in the clinical laboratory for which the tests were developed (Table 2).

These diagnostic tests are either (1) manual, which can take a trained laboratory technician several hours from sample processing to results; (2) partially automated, which utilizes specialized equipment to reduce hands-on time; or (3) fully automated, walk-away techniques, which require only minutes of hands on-time for sample loading, single-use cartridges for each patient specimen to be tested, and expensive, highly specialized equipment to perform all of the assays. Further, depending on the technology employed, these assays have estimated testing times from 50 minutes to 4 hours for the semi-automated to fully automated/walk-away assays, and 6-14 hours for the manually performed assays.

Point-of-Care (POC) PCR-based Lateral Flow assay and Isothermal NAAT. Currently there are 2 true POC molecular diagnostic tests available (Table 1). These tests can produce results in minutes, and therefore, can be done during a typical office visit for patients who have clinical symptoms and epidemiological risk factors for COVID-19. First, the Mesa Biotech Inc., Accula™ SARS-CoV-2 test (Mesa BioTech, Inc.; San Diego, CA) uses PCR to amplify a portion of the N gene, and

lateral flow along a test strip for detection of this amplicon. These reactions occur entirely within the Accula™ Dock or Silaris™ Dock and the estimated testing time, from sample collection to results, is 30 minutes. The test cassette contains a sample pad, where patient sample is added and PCR occurs, a conjugate pad with nucleic acids labeled with a chromatographic probe, which sits on a nitrocellulose membrane with lines coated with capture sequences that are complementary to the viral target and control sequences, followed by an absorbent pad. Lateral flow assays work by capillary action across the nitrocellulose membrane. Second, the Abbott Diagnostics Scarborough, Inc. ID NOW COVID-19 (Abbott Diagnostics Scarborough, Inc.; Scarborough, ME) assay is an isothermal NAAT to amplify a portion of the RdRp gene and uses fluorescently-labeled molecular beacons to identify these RNA targets. All reactions occur within the Abbott ID NOW™ instrument and the estimated testing time is 5-13 minutes.

These POC molecular diagnostic tests are very easy to perform, require little specialized training for the clinician, and produce rapid results to guide clinician care. However, these tests can only be performed on specific instruments and amplify a single genomic target of SARS-CoV-2, reducing their sensitivity and specificity as compared to traditional qRT-PCR based molecular diagnostics. Therefore, under conventional circumstances, the results of these POC must be validated using molecular diagnostic testing. However, in light of the severe shortages of tests for SARS-CoV-2, these POC molecular diagnostics are typically not validated by qRT-PCR testing.

Serological Tests

Sample Preparation for COVID-19 Serological Testing

The typical specimens used for serological testing of COVID-19 include serum, plasma, or whole blood. Serum and plasma are both the liquid, cell-free portions of whole blood. Serum is collected following coagulation of whole blood and does not contain clotting factors. Plasma is collected by centrifugation from whole blood that has been treated with anticoagulants and contains all the clotting factors of the blood, which is thought to make it less stable for long-term storage. Antibodies are proteins that can be found in all 3 specimens.

IgM, IgG, IgA in SARS-CoV-2

Antibody responses arise about a week following infection, with the multimeric IgM, as the first antibody arising, often concurrent with active infections. IgM, therefore, is often used as an indicator of newly acquired infection. IgM antibody responses are typically short lived, and wane as the infection is no longer active. IgG antibodies appear towards the end of the active infection and can persist for months to years following infection. IgA is associated with mucosal immunity and considered to be an important player in respiratory infections.

Scientists are still learning about the natural history of COVID-19; however, preliminary serological reports suggest that the SARS-CoV-2 antibody response follows this typical pattern, with a majority of patients seroconverting within 2-week following symptom onset and all patients seroconverting by one month after symptom onset.[32-34] In these reports, IgM and IgA are the first antibody isotypes detected 1-week following symptom onset, followed by IgG, which typically arise 2-weeks following symptom onset, as the patient's symptoms resolve.[32,34] Further, To and colleagues report a strong correlation ($R^2 > 0.9$) between anti-SARS-CoV-2-NP and anti-SARS-CoV-2-RBD IgG and neutralizing antibody titers, which are indicative of an individual's immunity to SARS-CoV-2.[33]

Tests and Their Principles Employed for Serological Detection of SARS-CoV-2

Serological testing involves detection of antibodies, typically IgM and IgG, against specific proteins of the pathogen, called antigens. In an individual suspected for COVID-19, the presence of antibodies for SARS-CoV-2 indicates history of exposure to the virus and does not indicate or rule out active infection or predict immunity to reinfection. Serological testing for detection of SARS-CoV-2 is appealing to clinicians because results can be obtained quickly and the presence of SARS-CoV-2 antibodies in convalescing patients indicates that the individual was able to mount an immune response to the virus, suggestive of some level of immunity against the virus. Therefore, seropositivity in an individual who has recovered from COVID-19, may make that individual a potential donor of convalescent serum/plasma for the treatment of other patients with active COVID-19 infections.[35] Seven clinical trials have been initiated or are being planned to evaluate the efficacy of passive immunoglobulin therapy for severe COVID-19 (NCT04261426 [China];[36] NCT04264858 [China];[37] NCT04332380 [Colombia];[38] NCT04332835 [Colombia];[39] NCT04323800 [US, Johns Hopkins];[40] NCT04325672 [US, Mayo Clinic];[41] NCT04333251 [US, Baylor]).[42] These studies will also provide insight into the use of serology to assess immunity to SARS-CoV-2 infection.

Currently, testing for active SARS-CoV-2 infection is the priority for diagnostic testing to identify and isolate patients, both at a national and local level. However, public health officials and researchers alike plan to conduct future seroprevalence studies, to determine the true prevalence of SARS-CoV-2 infection in populations.

Lateral Flow Immunoassay (LFIA). A simple POC serological diagnostic assay with results in minutes is the LFIA. Similar to the PCR lateral flow assay described above, a LFIA comprises of a test strip containing a sample pad, a conjugate pad containing viral antigen conjugated to a chromatographic tag (ie, colloidal gold, latex, fluorophore), which sits on nitrocellulose membrane with lines coated with capture antibodies against human antibody isotypes (ie, IgG, IgM, IgA), a control line, and

an absorbent pad. If a patient has had a past viral infection, their antibodies will bind to tagged viral antigen, and these antibody-tagged antigen complexes will bind to immobilized capture antibodies, which can be visualized as a line by the conjugated chromatographic tag. There are 3 LFIA with FDA EUA that can identify an individual seropositive for SARS-CoV-2 IgG and IgM antibodies in 15-20 minutes; the qSARS-CoV-2 IgG/IgM Rapid Test (Cellex, Inc.; Research Triangle Park, NC); the DPP COVID-19 IgM/IgG System (Chembio Diagnostic System, Inc.; Medford, NY); and the Anti-SARS-CoV-2 Rapid Test (Autobio Diagnostics Co. Ltd.; Santa Maria, CA) (Table 3).

Enzyme-Linked Immunosorbent Assay (ELISA). ELISA is the most common serological test used to detect the presence of antibodies specific to an antigen of interest in a patient serum. In an ELISA, viral antigens are immobilized on a surface, and then a patient's blood or serum is added, allowing for any antibodies specific for the viral antigens to bind, while all other antibodies are washed away. Then an enzyme-conjugated secondary antibody, specific for the antibody isotype being targeted (ie, IgM, IgG, or IgA) is added, followed by the enzyme substrate, resulting in production of a colored product when viral antigen-antibody complex is detected. There are 4 ELISA with FDA EUA; the VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total and IgG Reagent Packs (Ortho Clinical Diagnostics, Inc.; Rochester, NY); the COVID-19 ELISA IgG Antibody Test (Mount Sinai Laboratory (MSL); New York, NY), only approved for use at the MSL; and the LIAISON SARS CoV-2 S1/S2 IgG (DiaSorin, Inc.; Stillwater MN).

Microsphere Immunoassay (MIA). The MIA is a variant of an ELISA which couples viral antigens to magnetic carboxylated microspheres and fluorescently labeled secondary antibodies to detect serum antibodies in antigen-antibody complexes. The microspheres are impregnated with dye that makes each microsphere a spectrally-distinct set or region, making this a unique technology that allows for multiplexing of several antigens, each coupled to spectrally distinct microspheres, within a single reaction. The different antigens may correspond to different proteins of the same virus or of different pathogens, allowing for differential diagnosis among a panel of pathogens that may present with similar clinical symptoms and/or co-circulate in the same geographic region.

Serological analysis by ELISA and MIA are much more sensitive and specific diagnostic tools as compared to lateral flow chromatographic immunoassays, however, the testing time required is at least 5 hours for ELISA, and 3-8 hours for MIA. The MIA is a newer technology that requires more costly reagents and highly expensive equipment to perform as compared to an ELISA.

In addition to these 7 serological tests which have received FDA EUA, there are numerous commercial manufacturers and high complexity CLIA laboratories that are working on or have developed their own LFIA, ELISA, and MIA based serological tests for the detection of SARS-CoV-2 antibodies.[43] While none have received FDA EUA, internally validated tests may be used for clinical diagnostics under Section D of the FDA's March 16, 2020 expanded COVID-19 diagnostic guidelines.[7] Many of these tests are likely to receive FDA EUA in the next weeks to months as transmission in the US subsides and the need emerges for public health programs and researchers to conduct community-wide serological screening studies of SARS-CoV-2 asymptomatic and symptomatic infection.

Conclusion

As of April 25, 2020, the FDA has granted EUA for 48 COVID-19 in vitro diagnostic tests and 21 high complexity molecular based laboratory developed tests.[6] Most of these tests work on the principle of identifying specific regions of the SARS-CoV-2 genome, primarily the ORF1ab, RdRp, S, E, and N genes. Clinical understanding of SARS-CoV-2 and COVID-19 is evolving day-by-day; however, from what is known now, it is important to include gene targets highly conserved across all coronaviruses, such as non-structural proteins, as well as gene targets with some diversity between the different coronaviruses, such as the structural proteins, to ensure high levels of sensitivity and specificity. Patients with positive results from these tests, along with clinical signs/symptoms and/or epidemiological factors consistent with COVID-19 can be considered as presumptive positive cases of active SARS-CoV-2 infection. Four serological tests, identifying SARS-CoV-2 specific IgG/IgM antibodies have been granted FDA EUA. Positive results of these serological tests indicate recent infection with SARS-CoV-2, and these results, along with ongoing research into the natural history of COVID-19, could be useful in determining the susceptibility of an individual to future SARS-CoV-2 infection.

Aside from these diagnostic tests with FDA EUA, in order to address national diagnostic shortages, the FDA enacted a new policy on March 16, 2020 regarding guidance for COVID-19 diagnosis during the public health emergency.[7] This policy has allowed high complexity CLIA laboratories and commercial manufacturers to use diagnostic kits developed internally with proper validations in place for clinical testing prior to FDA review of EUA applications. Patients must be informed that although these diagnostic tests have been internally validated, independent review by the FDA is pending. Further, this new policy has also allowed for expanded use of SARS-CoV-2 serological testing without EUA. Here also, patients must be informed that the FDA has not reviewed the test.

Conflicts of Interest

The authors report no conflicts of interest.

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