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A Review of the Current Testing Methods for SARS-COV-2 in the US

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Introduction

On March 11th 2020, the WHO classified the Covid-19 out-break as a pandemic¹. As of May 10th 2020, according the WHO Situation Report-111, there are 3,917,366 confirmed cases of Covid-19 with 274,361 deaths globally². The estimated basic reproduction number for Covid-19 is 2.2, meaning on average a person with the disease will infect 2 other people³. As of the date this paper was written, there are no conclusive methods of treatment, nor a vaccine for Covid-19⁴. It is because of this that, arguably, the most effective policy to preventing deaths, harm, and extending isolation policies, is with mass tracking of current cases, followed by rapid testing of populations exposed to said individuals⁵.

The United States of America has taken a triage approach to testing however, with classifications of people being designated as those who should receive testing while others are not. A list of symptoms⁶ is first used to identify if a patient could have Covid-19. Other risk factors such as, exposure to a person with a confirmed case, if they work in a high-risk area, or if their local area has a high exposure rate are used to judge if a test is required. The CDC provides official guidelines on classification of risk assessment for testing⁷.

In general, for the majority of the public, no testing would be allowed unless the individual is seen by a medical professional as having all the listed symptoms and at least one other risk factor, although there have been many reported instances of people without symptoms gaining access to tests⁸. For most people who are showing symptoms related to Covid-19, the response is the same, self-isolate, call a medical professional if symptoms get worse, and call a healthcare professional if the person complains of shortness of breath, trouble breathing, or any escalation of symptoms⁹. Transmission of Covid-19 can occur with people who are asymptomatic though, which means that the virus cannot be stopped by just having people showing symptoms isolate themselves. If we want to stop the spread, we must get in front of it by tracking and testing as many people as we can.

There are two types of tests that can be conducted, viral, and antibody. Viral tests are used to detect if there is any live virus inside a patient's body at that moment¹⁰. It is mostly used

in point-of-care (POC) situations, meaning at hospitals, physicians offices, or as will be shown later, at the home of the patient. They come in a few different types but mainly as real time reverse transcription polymerase chain reaction (real time RT-PCR) or real time qRT-PCR¹¹. Antibody testing can be done in a POC or a lab setting. Antibody testing cannot tell whether a patient currently has the virus, as it uses the presence of antibodies to tell if a patient might have had the virus in the past, since the bodies immune system would have developed the antibodies to fight the virus¹². Tests are usually run by real time RT-PCR, Lateral Flow, enzyme-linked immunosorbent assay (ELISA), or in one example, field-effect transistor.

1. Viral Testing

Since most people that will require testing are those who are currently showing symptoms or work in high risk areas, viral testing has taken a front seat in the fight against Covid-19. As of February 4th, 2020, nearly 54 viral testing kits have been approved by the FDA under the Coronavirus Disease 2019 (COVID-19) Emergency Use Authorizations for Medical Devices policy, instituted in order to rapidly move new technologies from the testing stage to use in the field¹³. It is important to note that the only other tests on that list are antigen/antibody tests. These tests include standard real time RT-PCR test, and two newly approved at-home sample collection tests with dedicated facilities for analyzing said tests.

The first at home viral test uses the new patented LabCorp's Pixel by LabCorp COVID-19 Test home collection kit^{14 15}. The kit includes a nasal swab and saline for collection along with a container to put the sample in. Once collected, the sample is sealed and shipped to an approved LabCorp facility where it is analyzed using RT-PCR and results are sent back to the patient. The kit requires a doctor's approval to order, or by filling out an eligibility survey located on the site linked previously. Time to results is not listed anywhere visible, however, one could assume that it would vary based on the workload of specific testing sites.

The second at home viral test uses the new patented Spectrum Solutions LLC SDNA-1000 Saliva Collection Device, paired with analysis at Rutgers Clinical Genomics Laboratory using the high complexity molecular based LDT "umbrella" that was previously approved by EUA¹⁶. Unlike the previous test kit, this one collects saliva samples instead of requiring a nasal swab. This is beneficial as cotton swabs can become scarce during emergency situations such as pandemics, and cotton swabs themselves can interfere with RT-PCR since the separation of the RNA sample from them can lead to errors in results¹⁷.

The approval of the SDNA-100 Saliva Collection Device was approved based off a study by multiple departments at Yale University including the Department of Epidemiology of Microbial Diseases, Department of Medicine, Section of Infectious Diseases, Department of

Immunobiology, and many more¹⁸. The study, titled “Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs”¹⁹, claims that by using saliva to get RNA, instead of nasopharyngeal swabs, “titers from saliva were significantly higher than nasopharyngeal swabs ($p < 0.05$, Mann-Whitney test).” They also claim to have detected SARS-CoV-2 using the saliva but not the nasopharyngeal swabs in eight patients, while only detecting SARS-CoV-2 using the nasopharyngeal swabs but not saliva in three patients¹⁹.

The study goes on to further claim that during longitudinal studies with sample’s being collected on average every 2.9 days, “we found 5 instances where a participant’s nasopharyngeal swab was negative for SARS-CoV-2 followed by a positive result during the next collection (5/33 repeats, 33%)¹⁹. However, with the saliva samples, “there were no instances in which a sample tested negative and was later followed by a positive result.”¹⁹. Since hospitals have limited numbers of beds available, knowing when a patient can be sent home can be critical to providing resources for other patients. A false negative result could end up with the patient being discharged before they are clear, which could mean the patient infects other people, resulting in more people going to the hospital.

Although these results seem promising, the author’s note that they have limited data to support the claim that saliva could be more sensitive for detection purposes. The sample size used (121 participants¹⁹), was not large enough to conclusively prove the hypothesis and that they would require a larger sample size for full confirmation. Also, the findings have not been peer-reviewed yet. This is a preprint of the results, and although promising, giving the procedure EUA status may not be in the best interest of patients, since there are proven testing methods already in place. It is up to the reader to decide if the results are promising enough to trust using the kits, however, this author found that most articles written about this method only mention that the study used to justify it has not been peer-reviewed at the end of such articles if at all¹⁶.

2. Antigen/Antibody Testing

Antigen/antibody tests, while not as widely used as viral tests, are still critical to stopping the spread of Covid-19. From the Centers for Disease Control: “CDC is looking at data from antibody tests to estimate the total number of people who have been infected with SARS-CoV-2 in the United States. CDC is also using antibody testing to learn more about how the body’s immune system responds to the virus and to explore how the virus spreads among people exposed to it.”²⁰. These tests are usually conducted using blood samples drawn from volunteer patients, and use ELISA, lateral flow, or PET method to determine the presence of antibodies for verification on if a patient has likely had the virus. This can be accomplished because of the publication of the complete coronavirus genome²¹. SARS-CoV-2 antibodies were then developed with high binding affinity to one of the four main structural proteins that Covid-19

encodes for, spike, envelope, matrix, or nucleocapsid. Specificity towards SARS-CoV-2, and not SARS-CoV or MERS were carried out²².

Products that use this antibody include the Anti-SARS-CoV-2 IgG ELISA²³. This product could not be found on the manufacturers website at the time of writing, though this could be due to the fact that FDA approval occurred on May 5th, and writing of this paper finished on May 11th, so the website could be updated since then, here is another manufacturers version²⁴. ELISA works by detecting IgG levels in patient samples, which are consistent with the body's immunological response to Covid-19 infection. The test requires incubation before results can be determined, so result time can vary depending on a myriad of factors including patient sample purity and workload of designated facilities.

Whereas the previous test looked for IgG levels in a patient's blood, Quidel's Sofia 2 SARS Antigen FIA tests for the presence of Covid-19 through binding of the nucleocapsid protein into specific areas in the machine itself²⁵. After taking a nasal or nasopharyngeal swab of the patient, the swab is inserted into a Sofia 2 instrument. The lateral flow Test Cassette will separate the Covid-19 RNA from other specimen's present, and using anti-SARS antibodies, will bind to the nucleocapsid protein and emit a fluorescence. The level of fluorescence can then be used to determine if the patient has had the virus. Some downsides to this test are that it is not specific to SARS-CoV-2, and will also detect SARS-CoV. However, unlike other antibody testing, this device can be used in POC environments, and only takes about 15 minutes to have results²⁵. The tests are available though the Sofia 2 machine itself is sold separately²⁶.

A third antibody test which is still up for FDA approval is the Field-Effect Transistor Based Biosensor. In the paper "Rapid Detection of COVID-19 Causative Virus (SARS-CoV-2) in Human Nasopharyngeal Swab Specimens Using Field-Effect Transistor-Based Biosensors" by Giwen Seo et al. the authors report that a Field-Effect Transistor (FET) can be used for rapid detection of Covid-19 antibodies²⁷. Unlike the previous two tests, this one uses specific binding of the spike protein in Covid-19 to detect for its presence. Using potential differences on an activated graphene base, this test could provide rapid results even in a POC environment. The device does not require any separation of and purification of RNA, unlike real time RT-PCR, ELISA, or lateral flow methods. Increased sensitivity was also reported using this method as opposed to ELISA²⁷. Further testing is required however, as the sample size used during the study was very small (4 patients total), and other factors such as durability of the test under multiple conditions and run-throughs must be determined.

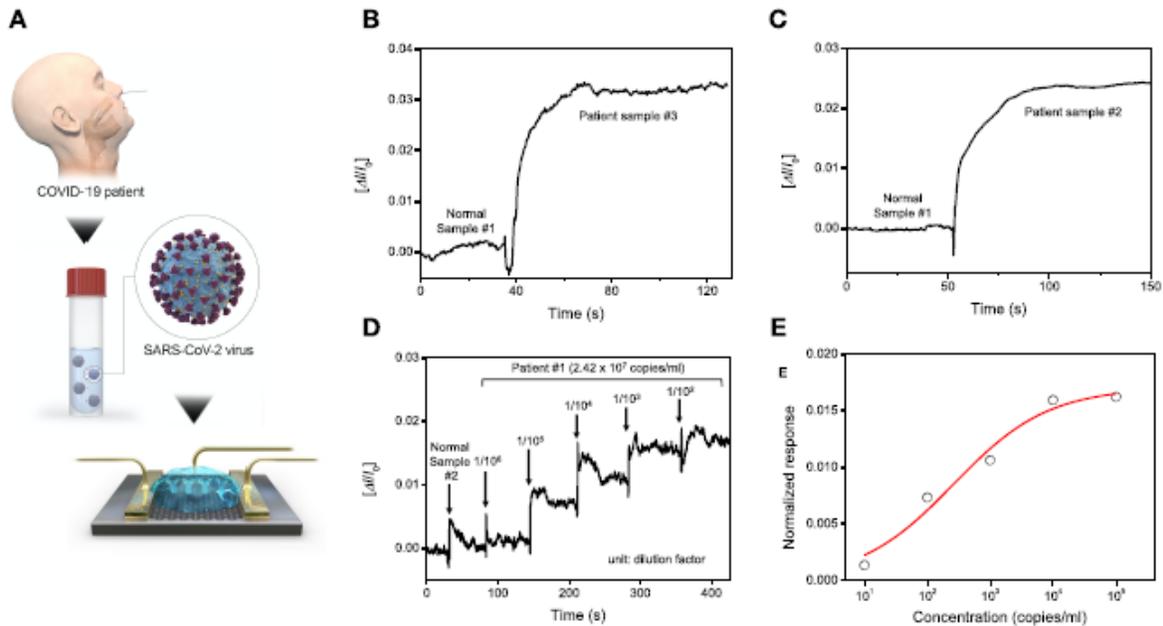


Figure 1. adapted from ref 27

Conclusions

Tracking outbreaks, along with rapid and mass testing of patients for Covid-19 is paramount to stopping the spread of the virus. Multiple testing kits have been invented, all working under different conditions but with similar results. A main area that must be tackled is the false reporting of how accurate such tests are and for what reasons. Literature suggests that, given a lack of test accuracy reporting and a general lack of scientific knowledge, that Covid-19 tests are inaccurate. The truth is that, while mistakes can be made in multiple steps, such as sample collection, purification, and in the machines themselves, a vast majority of these tests are accurate. A negative antibody test, for example, does not mean that a person has never had the virus, as antibodies can take a few days after infection to show up on results. At the same time, a positive result on an antibody test does not mean that a person currently has the virus. A positive result on a virus test does indicate that a person currently has the virus, but a negative result does not mean the patient is in the clear, or that they cannot contract the virus later. Testing is done for specific reasons in specific conditions. A patient should consult their doctor about testing and the results to get the full story. Just because you know someone who tested negative and then died of Covid-19, however, does not mean the tests are inaccurate. An understanding of FDA approval of testing kits is also important. Just because the tests have not had full official FDA approval does not mean they are inaccurate; it just means they have not gone through a full rigorous approval. Misinformation about testing can lead to patients not getting tested for fear of false positives or false negatives. This can further lead to them infecting other people because they believe they do not carry the virus. Good reporting, such as this article published by NPR are what is required to properly inform people about testing²⁸.

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