An Optimized Method to Identify the SARS-CoV-2 Virus in Students at the University of Maryland, College Park: Comparisons with the Current State of the Art

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Introduction

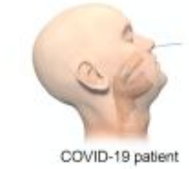
As of July 2020 the SARS-CoV-2 virus has infected 4,169,991 Americans and killed 147,333[1] of those infected. COVID-19 is the name given to the disease contracted from this virus, a novel beta coronavirus. As this virus continues to spread and kill in the United States it has sparked many debates about how to handle the reopening of public and private schools in the Fall. As an undergraduate at the University of Maryland, College Park I know that my school and state are taking the current pandemic very seriously and as such only about 20% of classes will be in person with the rest being online. To attend these classes the only requirement is to wear face coverings and to take your own temperature and report if you have a fever or not. In addition, I know that even though most classes are online the majority of students and staff will be returning to campus or housing within walking distance of campus come August.

While I am sure the majority of students will take all the necessary precautions to prevent the spread of this highly dangerous virus it is still college and students will want to party and feel invulnerable to the virus while doing it. Also, there is no way to ensure 0% transmission between such a large and diverse population like the one enrolled and employed at the University of Maryland. The school has already employed PCR tests from July 14-15, 2020 and managed to collect nasal swab samples from 1,800 individuals and evaluate them within 72 hours. This is impressive but PCR tests have limitations that may make them less than ideal for the 30,762 student undergraduate population[2], not to mention any additional staff returning to campus. These limitations include low sensitivity, false negatives, being laborious and time-consuming to perform, and requiring special equipment.[3] Fast results and sensitive tests are invaluable as they work to swiftly quarantine and treat an infected individual before they can spread a virus that on average will spread to 2.2 more people[4], by doing what people in college do.

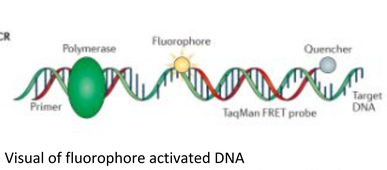
This project sought to address the problem of testing a large student body migrating from around the nation for SARS-CoV-2 in the most effective and accurate way possible. After a thorough literature review of proven assays for this novel coronavirus a combined approach of a Reverse-Transcription Loop Mediated Isothermal Amplification (RT-LAMP) Assay will be supplemented by a Field-Effect Transistor (FET) Assay.

Methodology

There are various methods that can be leveraged to detect SARS-CoV-2 in patients. The bulk of my RiSE project consisted of seeking and understanding not only the nature of viruses but the many assays used to detect them in humans. Foremost, among those used to detect the novel coronavirus is a process known as reverse-transcription polymerase chain reaction (RT-PCR). This process uses an enzyme known as reverse transcriptase to generate complementary DNA from an RNA template. The DNA is then amplified by 1) alternating heat cycles, 2) flooding the solution with primers, and 3) allowing a DNA polymerase to add complementary bases to each base on the single stranded DNA until 2 double stranded DNA fragments are obtained, where the process is repeated.[5] DNA-specific dyes are used to identify the target DNA through fluorescence. This technique, in "real time" format, means that DNA can be analyzed as it is amplified, is the current gold standard for detecting SARS-CoV-2. Another widely used method are antibody tests based on ELISA (Enzyme Linked Immunosorbent Assays). Essentially, each plate on a 96+ well plate is functionalized for antibodies specific for the viral antigen proteins, which will then be functionalized onto the bound antiboudy. A second set of specific primary and secondary antibodies will then recognize and bind to the target protein analyte, and, upon addition of a substrate, a color change will be observed. Through methods like fluorescence it is possible to measure the intensity of this color change and to correlate it to the concentration of antibodies in the patient. These tests can be used to identify both the body's antibody response, typically immunoglobulins G and M. Antibody testing and real time RT-PCR represent the bulk of testing methods but many more efficient and accurate methods have successfully been used to identify SARS-CoV-2 in patients.

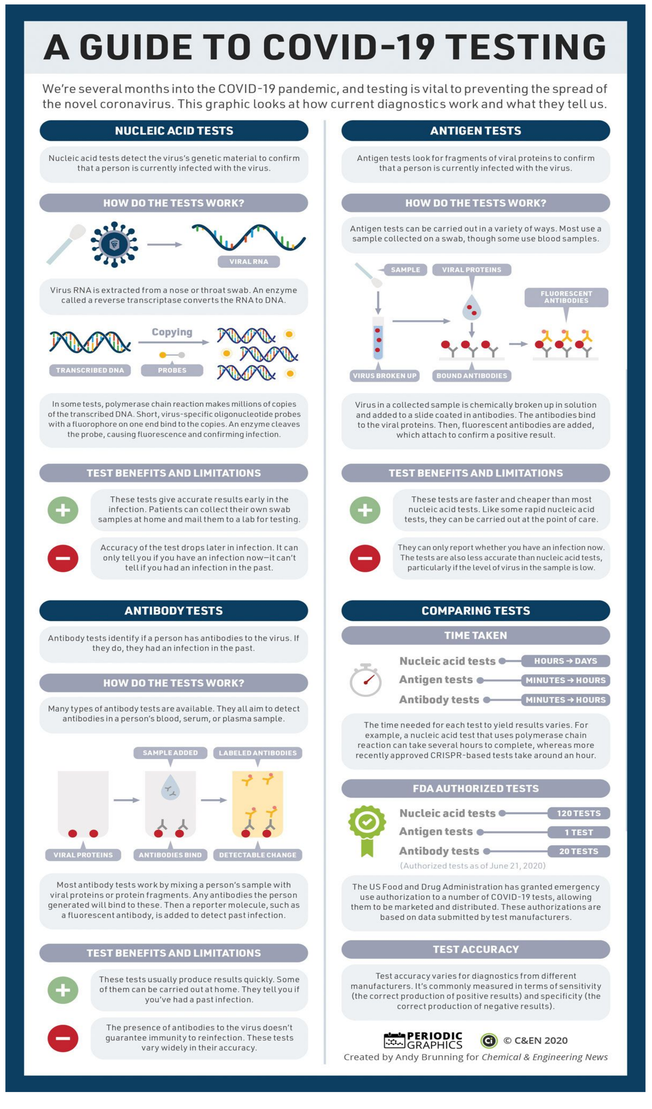


COVID-19 patient



Above: Visual of fluorophore activated DNA
Left: A nasopharyngeal swab, through which samples for testing are usually collected. Samples suffer from low viral loads as SARS-CoV-2 tends to rest in the lower respiratory tract, requiring lower limits of detection.

State of the Art



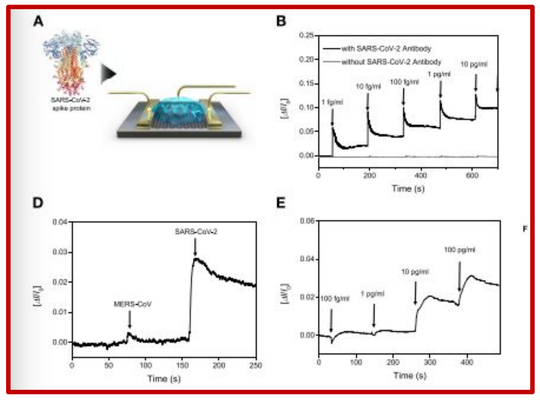
RT-qPCR has been a successful and widely used test since the earliest months of the SARS-CoV-2 outbreak. It has a low limit of detection for viruses and is very specific. There are drawbacks to this technique. It takes a long time to complete the assay, it is difficult to administer, requiring highly trained technicians, a large lab space and very specific equipment are needed, high temperatures need to be sustained and cycled which is costly, and it suffers from false negative results. ELISA based assays are useful for detecting the virus a week after infection and on. These assays can detect antibodies produced even after symptoms have gone away and the viral load becomes undetectable. They are also relatively easy to perform and analyze at a low cost. Antibody assays suffer from the fact that they can only detect the virus after the body responds to it, meaning infected individuals can still spread the disease for many days before it is identified. Also, these tests have higher limits of detection than most other assays. Although these "staple" detection methods have served the global community well there currently are proven tests that address their drawbacks to provide accurate, more rapid, and cost effective testing platforms.

Proposed Approach

The workhorse of my proposed approach would be a RT-LAMP based assay. LAMP is a technique for amplifying DNA that operates at a constant temperature and produces much more DNA than PCR. The specific RT-LAMP methodology that would be employed by this approach utilized 5 different primers to amplify the six regions of the target orf1ab gene of SARS-CoV-2[3]. It would be run at 63^{0}C and presents positive results in clinical samples with the orf1ab-4 primer within an hour.[3] Test results are as easy to determine as observing if the test solution turns green for positive or remains orange for negative, both observable with the naked eye.[3] This assay showed significant more sensitivity than the RT-qPCR also tested in the study with the LAMP assay providing positive results at as low as 20 copies per reaction compared to the PCR assay which was sensitive only to 200 copies per reaction.[3] This test required a single step at a constant temperature rather than several steps at varying temperatures (which requires an expensive piece of equipment known as a thermocycler), and used a small instrument instead of a large lab full of expensive equipment. This RT-LAMP assay was shown to be both 10% specific and sensitive and required a shorter time than PCR to produce clearly visible results, demonstrating its superiority as a detection method for SARS-CoV-2. Still, there is a far more sensitive detection method which would undeniable confirm if a patient has the virus or not.

Field-effect transistors can detect current flow from a source to a drain as charge carriers pass through. Channel conductivity is measured at the gate which rests between the source and drain. FET can be used as a biosensing device by immobilizing viral antibodies onto the gate and measuring potential. Once a calibration curve has been established the unique relationship between viral concentration and potential can be established, allowing researchers to determine the amount of antigen in the sample. This particular use of FET as a biosensor had SARS-CoV-2 spike proteins immobilized on a graphene surface.[4] Using this tactic novel coronavirus antigens were detected down to 100 fg/mL within the clinical transport medium.[4] Unlike RT-LAMP no RNA processing step is required as FET detects the virus directly. This greatly increases the speed of the test. This study showed that this particular use of FET as a biosensor was extremely quick and sensitive. In my proposed approach this technique would serve to supplement RT-LAMP assay as it is accurate and fast and requires little prep. Also the FET technique requires very small samples so the nasopharyngeal swab would not have to be taken again. It would serve in only a supplementary roll as the methodology is more complex and analysis is less straightforward. As PCR test are already being used at UMD, the necessary equipment for RNA extraction is already present so the fact that FET does not require this step while RT-LAMP does is less of an issue.

Both of these techniques are extremely accurate while being faster, and cheaper than the current standard test, RT-qPCR. It is my hope that these two methods of detecting SARS-CoV-2, one identifying the viral RNA and the other confirming the presence of the virus's most identifiable protein, will work in tandem to protect the large number of students and faculty returning to the University of Maryland at the end of August. This proposed approach will have infected individuals swiftly quarantined so that the virus will stay contained and the university can function as it has set out to during this pandemic and will fully reopen as soon as possible.



A) Schematic of the FET detector and the spike protein it's detecting. This protein uses its spikes to readily bind to the cellular entry responder that leads to infection.

B, E) FET readouts showing how personnel would determine the presence of the protein in varying concentrations.

D) FET readout demonstrating the specificity of the method between even closely related coronaviruses.

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