COVID-19 Alternative Testing Analysis

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Testing Analysis and Comparison

Right now, labs across the world are using a diagnostic test based on a set of procedures called polymerase chain reaction (PCR). This method was invented in 1985 and won a Nobel prize.[1]

The basic idea is to take a sample from the patient, isolate and duplicate any viral RNA/DNA in the sample, and then use a fluorescent pigment intercalated with the DNA or a pH indicator to determine the amount of at least the presence of viral RNA.

[1] More specifically, they are using the realtime RT-PCR or qRT-PCR tests, which just means that firstly, the amount of the virus is measured continuously throughout the test (in "real-time"), and since SARS-CoV-2 is an RNA virus, RT means that the test simply uses the complementary DNA (cDNA) to the RNA strands.

This method is slow, expensive, complex, and at least for COVID-19, there are concerns about its accuracy for detecting the virus.[2]

In this article, I first describe the issues with our current testing method, and then how an alternative method called RT-LAMP is a marked improvement in every one of those areas.

RT-LAMP stands for reverse transcriptase loop-mediated isothermal amplification and is a variation on the LAMP technique.

I may write a separate article describing these and other types of testing methods there are. However, these are the only two molecular assays that are in real use for testing COVID-19.

Let's address each of these concerns individually.

Time

In diagnostic testing for a pandemic, most experts would likely say that throughput is more important than the absolute time it takes for each test. However, time is still important to the patients, and in many cases a shorter absolute time allows for greater throughput.

According to ThermoFisher, one of the leading providers of qPCR tests, the average running time for a PCR test is 2 hours. They also market their "Fast PCR" tests, which cut that down to 60 minutes. ("Benefits of FAST Real-Time PCR - US" n.d.)

The most experimental research for PCR tests by using a micro-sized version of the test can do 40 cycles of PCR in 6 minutes. However, this can only process 100 nanoliters of a sample, and would require many in parallel to gain throughput capabilities. (Neuzil et al. 2006)

LAMP tests can be completed, after the pre-processing steps, in less than 20 seconds.

Cost

qPCR tests require large, expensive machines called thermocyclers, along with reagents and other laboratory equipment necessary to extract and prepare the DNA or RNA carefully before the test.

Some groups have shown that they have been able to decrease the "cost per reaction" of PCR tests to around a dollar. (Santos et al. 2017)

However, the main issue here is the initial capital outlays for buying the requisite equipment, which may not be a problem for existing labs in developed countries,

[2]This is why China had a large bump in their number of reported cases: because they changed the criteria to patients who had the symptoms, not just those who tested positive on the test. In fact, many cases will test negative multiple times. See the Accuracy section.

but can be an issue for remote locations.

This is why LAMP tests have become popular as a more accurate form of rapid diagnostic tests (RDTs) for some diseases such as malaria and tuberculosis which are major issues in many developing countries.

Complexity and use of Healthcare System Resources

Not only do qPCR tests have to be done in laboratories, but they also have more specific requirements for the samples that need to be collected, and how they are prepared.

Firstly, the PCR process, because it can be used for more advanced procedures, requires careful preparation of the sample in the lab, which can lead to shortages of requisite reagents, as has been widely reported.

However, LAMP tests have been shown to work directly on samples with little or no preparation. In fact, during the Zika virus outbreak, a RT-LAMP test was developed that could test individual dead mosquitoes by simply placing them in a test tube with water, and then running the test.

For example, for COVID-19, most testing facilities use a nasopharyngeal swab which is uncomfortable and sometimes painful as a healthcare practitioner sticks a swab down one's nasal cavity, often eliciting coughs or sneezes that could contain the virus. This is why healthcare workers need to wear and change into new personal protective equipment (PPE) before every test.

This leads to real challenges, including the simple time added for healthcare workers to properly clean or change their PPE before a test, and if the supply chain cannot provide adequate PPE for doctors and others who need it.

For example, healthcare workers at drive-thru testing stations using N95 masks may be preventing a doctor in a hospital from caring for a critical-condition patient because of a lack of masks.

LAMP may allow for alleviating this problem by allowing for patient-collected samples.

Studies have given mixed signals about the effectiveness of patient-collected samples and there are concerns about their uniformity. However, for most respiratory diseases, all indications suggest that patient-collected samples using saliva or a nasal swab (which is much easier and less uncomfortable) is just as accurate as nasopharyngeal swabs.

Additionally, the tests themselves generally have to be performed by highly trained workers.

Accuracy

qPCR testing is considered the "gold standard" for DNA and RNA quantification, and is referred to as such in many papers, mainly because of its use in the scientific community for 35 years.

However, during the outbreak of this Sars-Cov-2 virus, there have been concerns about the accuracy of the specific test for COVID.

Firstly, studies have shown that asymptomatic cases and even symptomatic ones may test negative multiple times, but still be clinically diagnosed with COVID-19 for other reasons (blood tests, CT scans, etc). (Hu et al. 2020)

Additionally, even in symptomatic and severe cases, only 59% of suspected COVID cases were detected via PCR tests, and up to 33% of negative PCR tests were clinically diagnosed as having COVID. Thus, CT scans are recommended for more comprehensive diagnosis in endemic areas. (Ai et al. 2020)

Thus, there are concerns about the accuracy of PCR tests for COVID. More retrospective analysis of procedures, sample, etc will be required to determine the issues with this test.

Based on published papers, RT-LAMP is at least as accurate, if not more accurate than PCR for COVID testing.

Again, further analysis is needed to figure out possible reasons for this.

So far, this article seems rather one-sided by presenting issues which are important, and I have tried to discuss objectively, but seem to favor LAMP.

So: Are there benefits to PCR?

Of course.

PCR is generally more amenable to complex experiments and new research that require exact control over DNA.

It can be used for complex biological procedures such as "genotyping, cloning, mutation detection, sequencing, microarrays, forensics, and paternity testing."

However, for simple diagnostic testing, where the goal is to detect whether or not the pathogen exists[3], it seems that using the best tool for the job, and the tool that can scale to provide requisite capacity, even if it is not through conventional labs, makes the most sense.

[3]This process consists of amplifying the RNA (in this case) such that it can be detected by a fluorescent gel or a change in pH, but with some constraints. For example, such that the test is **specific** and not **cross-reactive** (doesn't detect similar viruses like SARS, MERS H1N1, etc). Additionally, the test should have a high **sensitivity** and **low level of detection** (e.g. it can detect the virus if only 10 genome equivalent copies of RNA are present in a sample)

Conclusion

Based on these main areas of concern, RT-LAMP is a much better candidate for large-scale deployment of COVID-19 testing.

I believe that it will be a vital tool for testing in less developed countries.

As we have seen a lack of testing capacity in the US, I also believe augmenting our existing testing system with some of the deployment possibilities outlined in the section below would be greatly beneficial.

Additionally, I believe that all laboratories in the US should at least evaluate ways to improve their capacity by deploying RT-LAMP in some way.

For some patients, performing a LAMP-based test and a PCR-based test might be valuable, especially to study their accuracy in more detail. Some studies will also use antibody-based detection methods like ELISA.

I hope that some labs will study the possibilities for using different samples in detecting COVID, for example, the differences between nasal swabs, saliva, urine, etc.

Additional Thoughts

Large-scale deployment options for RT-LAMP

This testing method could be deployed at a large scale in a variety of ways.

Firstly, we would want to enable patients to self-collect samples in their homes. This would allow patients to preemptively self-quarantine, receive the sampling tools in the mail and perform their own test.

One method is to send the entire testing apparatus to the patient. There has been some research on 3D-printed testing devices, and even a test that runs in a thermos, because LAMP requires the test tube to be heated to 60-65 degrees centigrade or 140-149 degrees F.

This would allow them to run the test for themselves as soon as they receive the device, delivering results within 30 minutes. However, the issue of heating the sample in a home is difficult.

Additionally, this might be more expensive due to shipping costs.

The other option is to have the patient collect their sample at home and mail it to a testing facility that could run many tests at once, possibly even thousands.

This could deliver test results soon after the sample is mailed in. Results could be delivered electronically and reported to the CDC at the same time.

This could be enabled by regional testing facilities similar to an Amazon fulfillment model, staffed by volunteers who follow basic standards for health and safety and are not in a high-risk group.

Regulatory issues

The tests and testing procedures would have to receive and Emergency Use Authorization (EUA) from the FDA.

All laboratories processing human samples are required to follow the federal Clinical Laboratory Improvement Amendments (CLIA) statute.

However, for simple tests[4] that use unprocessed specimens (like saliva or nasal swabs) and pose no risk to the patient, one can apply for a CLIA waiver, which then exempts the laboratory from inspection.

These facilities would likely be deployed on a regional basis in collaboration with state governments and governors. This could also allow for the use of National Guard personnel to operate the facilities.

Supply chain concerns

It doesn't seem to make sense to deploy entirely new facilities to scale up the throughput capabilities if they would lack the required reagents and other materials to run the tests.

However, I believe that the government could successfully figure out how to get the requisite materials, as they are generally less per sample than PCR tests.

This is an area where I have little knowledge, and so I would love feedback and help with this. If anyone could read the four existing papers on RT-LAMP and has experience with lab supply chains, it would be great to reach out.

At a national scale

It has been discussed that the lack of tests early in the process in the US was a "failing." The media has drawn comparisons to South Korea, which performed extensive testing and was thus able to better control the virus by using isolation rather than quarantines, and Italy, which in general didn't do as much.

However, there are reports of one town in Italy that tested all of its citizens, isolating the positive cases and then retesting periodically. The virus in that town ran its course and died out.

Another possibility is to test as many people as possible in the United States. One idea for encouraging this is to tie the cash check that seems to be a part of the proposed economic stimulus package to completing a self-test. This would then ensure with multiple rounds of data whether or not every American has the virus.

Of course, explaining the possibility that the tests aren't completely accurate is important as well.

[4] Tests where there is little to no possible human error by the operator of the test, and no medical judgment required to interpret the test.

Why aren't we doing this?

The question that I have asked myself throughout the process and especially as I have concluded this research is: Why does our developed health-care system feel the need to stick with this type of test when there is a better option out there?

This is the question I posed to David Walt and Pardis Sabeti of Harvard and the Greater Boston Consortium on Pathogen Readiness.

Dr. Walt's response was:

> The simple answer is there is a huge installed base of instruments based on qPCR. Other methods, such as RT-LAMP, are just not as ingrained in the existing infrastructure. For a crisis, the most expedient way to implement assays is to use the one that is most widely accepted. I suspect there will be big changes going forward but for now, the community is relying on what is easiest to scale.

Most labs are happy with the tests they have, even if they recognize that on a national and global scale, our testing resources are "a failing."

The truth is that some companies (about 4 startups) are selling at-home tests, however, they are likely not RT-LAMP tests which I believe could be scaled up much more.

Call to action: What can you do?

I urge you to contact your representatives at all levels of government to ask them about deploying new testing methods.

Additionally, I hope you share this article, or other resources describing new testing methods, with friends, family, and colleagues.

The Foundation for Innovative New Diagnostics has a list of all diagnostic tests for COVID-19: https://www.finddx.org/covid-19/pipeline/

This list includes companies and groups working on a variety of tests.

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