

# COVID-19 Alternative Testing Analysis

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## Introduction

The COVID-19 pandemic is likely the single greatest threat to public health we have ever seen.

Although many countries have taken aggressive and sufficient steps to control the outbreak, other countries, including the U.S. and Italy, have fallen behind.

In this paper, we compare the most widely used existing testing methods to newer state of the art methods that have massive potential for better scalability and throughput, in both developed and developing countries.

## 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.<sup>1</sup>

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.

This method is **slow, expensive, complex**, and at least for COVID-19, there are concerns about its detection accuracy.<sup>2</sup>

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 transcription loop-mediated isothermal amplification** and is a variation on the LAMP technique.<sup>3</sup> It is the most popular and widely used form of Isothermal Nucleic Acid Amplification Technology (iNAAT), which in general is more cost-effective and energy-efficient than PCR-based methods.

Let's address each of these concerns individually.

### Time

In diagnostic testing for a pandemic, most experts would likely agree 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*).

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 the requisite throughput capabilities (Neuzil et al.).

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<sup>1</sup>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.

<sup>2</sup>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. Many cases can test negative multiple times. See the Accuracy section.

<sup>3</sup>Parida et al. provides a valuable description of the process, although it is out of date by now. Additionally, Becherer et al. provides a modern review of sequence-dependent detection

LAMP tests can be completed, after the pre-processing steps, in less than 20 seconds (“LAMP-COVID-19 Kit”).

## 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.).

However, the main issue here is the initial capital outlays (~\$2000-\$4000) for buying the requisite equipment, which may not be a problem for existing labs in developed countries, but can be an issue for remote locations. Wong et al. and others have attempted to build thermocyclers specifically for remote locations.

This is why LAMP tests have become popular as a point-of-care (POC) tests for some diseases such as malaria and tuberculosis in many developing countries.<sup>4</sup> Tambo et al. showed that LAMP could be 30% more sensitive than the rapid diagnostic tests (RDTs) which they had used previously.

## 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, for DNA/RNA extraction, 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, various LAMP tests were developed (Silva et al.) that could test individual dead mosquitoes by simply placing them in a test tube with water, and then running the test.<sup>5</sup>

Next, let’s discuss the differences between types of sampling methods for respiratory infections specifically.

For COVID-19, most testing facilities use a nasopharyngeal swab which is uncomfortable and sometimes painful as a healthcare practitioner inserts a swab into one’s nasal cavity, often eliciting coughs or sneezes that could spread the

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<sup>4</sup>See the Background section of Vázquez et al., describing many previous papers applying LAMP in the field.

<sup>5</sup>For example, Yaren et al. was able to build a test for 3 diseases: the Zika virus, Dengue virus, and the Chikungunya virus in one test (known as a **multiplexed** test) simply based on unprocessed urine and blood plasma from humans, and a simple treatment protocol for the mosquitoes.

virus. This is why healthcare personnel (HCP)<sup>6</sup> need to change all or parts of their personal protective equipment (PPE) before each test (Health).

This leads to real challenges, including the *time added* for healthcare personnel to properly clean or change their PPE before a test.

It also poses a risk to the workers and the patients if they are either reusing PPE or using substandard equipment (like a bandana for a face mask) if the supply chain cannot provide adequate PPE due to the surging demand from doctors and others who need it (CNN; CDC).

Additionally, HCP at drive-thru testing stations or in a clinic performing sample collection 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 in a patient’s own home.

Studies have given somewhat mixed signals about the effectiveness of patient-collected samples in general. For respiratory diseases in particular, self-collection shows a lot of promise.

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 (Dhiman et al.).

Some other methods, like self-collection of throat swabs such as in Fisher et al., have shown not to add significantly to the sensitivity of detection.

For the SARS-Cov-2 virus specifically, preliminary research from Wang et al. has shown that the presence of the virus can be detected in many different type of samples.

Since this group analyzed data from patients at varying severity levels and with different viral loads, and since they did not have access to medical data to correlate as such, there are no real conclusions we can draw about the sensitivity, especially since they only collected samples from 8 patients for nasal swabs.

Further research is required in this area.

Additionally, the tests themselves generally have to be performed by trained workers, and some tests that include viral isolation must be run in a biocontainment facility.

## 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.

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<sup>6</sup>Also sometimes referred to in literature as healthcare worker (HCW).

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 and still be clinically diagnosed with COVID-19 based on other methods like blood tests or CT scans (Hu et al.).

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.).

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

Based on the existing published papers using RT-LAMP for diagnosing COVID-19, LAMP is at least as accurate, if not more accurate than PCR (Zhang et al.; Lamb et al.; Yu et al.; El-Tholoth et al.).

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” (*PCR (Polymerase Chain Reaction) LSR Bio-Rad*).

However, for simple diagnostic testing, where the goal is to detect whether or not the pathogen exists<sup>7</sup>, 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.

## 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.

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<sup>7</sup>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).

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° F (Kadimisetty et al.; Liao et al.).

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 an Emergency Use Authorization (EUA) from the FDA.

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

However, for simple tests<sup>8</sup> 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 with experience in laboratory supply chains would like to help, it would be much appreciated.

They could read the four existing papers on RT-LAMP for COVID-19, cited in the [Accuracy section](#) above and reach out to provide details on the potential availability or ways to substitute or mass-produce the reagents and equipment required.

## Discussion of Testing at a National Scale

Early on, Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases, characterized the lack of testing in the US as “a failing,” and since then, the media has tried to parse and understand why that happened (Chuck). However, based on what I've observed, even though there were errors with the original tests, it is mainly, as Dr. Fauci also described, because “we're not set up for” getting tests easily to people, a.k.a deploying testing on a large scale as described above.

He described how our healthcare system is optimized for a patient to doctor relationship and that all systems for testing are based on that system, where then a doctor would determine that a patient meets the criteria for a test, and then take the sample at that location and either

1. Run the test in their own hospital system
2. Send it to a state lab or the CDC lab

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<sup>8</sup>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.

3. Send it to a commercial lab company like LabCorp or Quest etc.

However, each of these locations is limited by throughput and has a limited ability to scale (COVID-19 Test Capacity Tracker). Although there were regulatory hurdles early in the process that limited these as some options, I believe the main issue still is throughput.

Obviously this poses challenges for people without insurance or with inadequate insurance, or people who don't have a regular doctor to go to.

This is an issue that has been discussed at WH Coronavirus Task Force briefings, and they have made a point to mention the addition of community testing facilities and drive-through facilities, which still usually have strict requirements.

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 ("Special Report").

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 (Tondo).

If we wanted to test as many people as possible in the United States we could tie the cash check that seems to be a part of the proposed economic stimulus package to completing a test. This would then ensure with multiple rounds of data whether or not every American has the virus.

However, at this point in the trajectory of the virus in the United States, it seems that the level of testing that we need is too little and too late.

Many experts agree that during the brunt of the pandemic, it is simply infeasible to test everyone that we want to, and even if we could, we wouldn't want to. Frieden provides an important outline of how responses to a pandemic must adapt at different stages.

At a certain point, if the virus has infected a critical mass such that it can't be contained by traditional surveillance measures, then social distancing must go into effect, meaning that testing doesn't result in a different outcome for many people, as they are already under quarantine or stay-at-home orders.

This is why in recent days we have seen states provide new guidance to prioritize the allocation of testing to only certain people (Johnson and Sun).

In retrospect, now that we understand the economic fallout of the pandemic better each passing day, it seems clear that it would have been worthwhile to spend even tens of billions of dollars weeks earlier if it enabled a South-Korea style situation with quick and effective testing and contact tracing, allowing for isolation instead of larger-scale quarantines.

This might have allowed us to avoid the type of massive shutdowns that experts agree are necessary for public safety when testing capacity is inadequate but are harmful to the economy.



Of course, explaining the possibility that the tests aren't completely accurate to people is important as well. Otherwise, people who receive a false negative result might return to work or social gatherings even if they are exhibiting symptoms or are at high epidemiological risk.

Unfortunately, even weeks later, we still know that we are not testing enough (Goodnough et al.; Rosenthal).

## 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, the two leaders of the [Diagnostics Working Group at 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.

## 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.

## Further resources

This article, along with my other research on COVID-19, is available at <https://covidtesting.alexkreidler.com/>

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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