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Many Initiatives Turning to RT-LAMP as Alternative to PCR for Rapid COVID-19 Screening Assays

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NEW YORK – To turn the tide on the COVID-19 pandemic, there is an increasing demand for large-scale screening and surveillance testing. A handful of commercial and non-commercial teams are now scaling up rapid SARS-CoV-2 screening assays incorporating reverse transcription loop-mediated isothermal nucleic acid amplification, or RT-LAMP.

A few commercial tests using rapid antigen technology have obtained Emergency Use Authorization from the US Food and Drug Administration, and their developers are ramping to manufacture millions of these tests per month. But LAMP, an equally fast and inexpensive technology that is also typically more sensitive than rapid antigen methods because it amplifies viral nucleic acids, hasn't been embraced in the same way by industry. Its application at scale seems to depend, at least for now, on commercial labs and the enthusiasm of grassroots efforts.

The LAMP technology was patented by Eiken Chemical in the early 2000s and has been licensed by many entities over the years, particularly for rapid infectious disease testing in low-resource settings. Typically, LAMP tests run in a 30-minute time range, which is a bit slower than antigen testing but a huge time savings over lab-based RT-qPCR.

Under a licensing agreement with Eiken, New England Biolabs advanced the core chemistry to incorporate pH sensitive dyes in 2016. The colorimetric NEB RT-LAMP test will turn yellow to indicate amplification, or remain pink in the absence of the target.

NEB supplies assorted RT-LAMP reagents for researchers to develop their own assays, and in July the Ipswich, Massachusetts-based firm launched a colorimetric RT-LAMP kit specifically for SARS-CoV-detection.

The essential elements of the NEB SARS-CoV-2 kit were first described in a *MedRxiv* pre-print in February, and in this early work scientists at NEB and collaborators in China validated the test in seven samples from patients in Wuhan.

Many groups have since independently ramped their own LAMP tests, some going so far as to create fully fledged screening programs. For example, computational genomics researcher Chris Mason and his colleagues at Weill Cornell Medicine began crafting an RT-LAMP assay in February, he said in a recent interview, and he has since scaled up the test to regularly surveil all city employees in Racine, Wisconsin, where his brother is Mayor.

For the most part, many of the earliest SARS-CoV-2 RT-LAMP assays to appear in the literature evaluated their tests on contrived samples. Such was the case, for example, for an assay from researchers at Beaumont Health System in Michigan that was posted to *MedRxiv* in February and subsequently published in *PLoS One* in June. An early test developed in South Korea and posted on *BioRxiv* in March and published in April in the *Journal of Molecular Diagnostics* also used contrived samples.

Researchers soon began publishing more validations of their rapid isothermal tests in small numbers of clinical samples. In April, a team in China published an RT-LAMP assay in *Virologica Sinica* that was validated on 24 clinical samples, while an assay published in *Microbial Biotechnology* was validated in 16 clinical samples and an assay published in *Emerging Microbes & Infections* in May was tested on 27 patient samples. In *Clinical Microbiology and Infection*, other researchers in China evaluated their RT-LAMP test on 130 clinical samples, reporting 100 percent sensitivity and specificity compared to an assay from BGI. The first three of these early studies used reagents from NEB, while the latter sourced reagents directly from Eiken Chemicals.

Lately, published RT-LAMP studies have begun describing even larger clinical cohorts.

First posted to *BioRxiv* in April, Mason and his collaborators validated an RT-LAMP assay in a cohort of 182 clinical samples collected from hospitalized patients in New York City.

More recently, a team in Heidelberg has described a trial involving 768 patient samples in *Science Translational Medicine*. The test used reagents sourced from NEB and had a sensitivity of 98 percent and a specificity of nearly 100 percent for samples estimated to have 1,000 RNA molecules present in the reaction.

And, a group at the University of Madison, Wisconsin screened saliva samples from 494 volunteers over 16 days with a mobile, RT-LAMP assay in a simple lab set up outdoors. The team published the results earlier this month in *MedRxiv* and it has also made the protocol for what it calls its "winnowing test" publicly available.

David O'Connor, a virologist involved in the Wisconsin RT-LAMP evaluation who specializes in HIV, commented in an email that, taken together, all the recent studies are pointing to basically the same conclusion — that "RT-LAMP is a useful complement to PCR-based nucleic acid diagnostics."

Simon Anders of Heidelberg said that his team was a bit surprised that RT-LAMP doesn't seem to have been used in many commercial diagnostic tests for other infectious diseases.

When the city of Heidelberg encountered a supply chain crunch, just as so many others have, Anders and his colleagues decided they needed to pioneer a method that would depend on alternate reagents.

"We ordered the New England Biolabs RT-LAMP kit and just tried it, and it worked immediately," he said in an interview. The group then developed "a proper test" to help screen city residents and is now talking with local authorities about scaling up testing for schools and workplaces.

Admittedly, the sensitivity of RT-LAMP can be lower than RT-qPCR, but the speed leads to higher throughputs, Anders said. And, the assay can be run by anyone with basic lab skills, so the team "purposely set it up with the idea that other university labs might want to copy it."

Anders noted that most tests use pairs of primers and probes, but RT-LAMP assay design is a bit trickier, because instead of one primer/probe set per targeted genomic region, an RT-LAMP test needs six or eight oligos. "LAMP primer design is a bit of an art," he explained.

The Heidelberg group decided to use primers and probes described in the early NEB study with a team in China, which target the virus' ORF1a and N genes. They found the N gene alone worked best, but the group is now also working on making improvements to the primer design. Others in the team have also hammered out a bead-based extraction protocol to increase throughput without the need for a liquid handling robot.

The RT-LAMP reagents from New England Biolabs seem to be incorporated into almost all assays described so far in the literature, and Steven Chiu, product marketing manager for NEB's DNA amplification division, said in an interview that the firm has been seeing increasing momentum for its RT-LAMP reagents.

The firm sells individual colorimetric LAMP components as well as optimized kits that include primers and controls for SARS-CoV-2 detection, Chiu said, so "people can either home-brew their own workflow and assay, or they can work with our kit."

Ultimately, customers may need to get a separate license from Eiken if they wish to use the LAMP reagents in a commercial diagnostic test, but NEB can serve as a go-between for that, Chiu said. "We have a really good relationship with Eiken," he added.

With all the different components, it is a little difficult to get to a price-per-reaction, he said, but the US list price ranges from $3 to $8. That price is often lower depending on the reagent format and volume purchased.

"We don't want price to be a deterrent for people considering colorimetric LAMP," Chiu said. "We are actively working with institutions and labs, particularly in third-world countries, to make sure that they have access to this technology," he said.

NEB claims it has adequate production capacity for all of this testing. The company is currently supporting high-volume requests to accommodate millions of samples within agreed upon timeframes, Chiu said. In the future, he thinks the RT-LAMP technology could fit with target product profiles of screening tests that will likely be needed in airports and by sports leagues.

Eiken makes a dedicated RT-LAMP SARS-CoV-2 detection kit as well. The kit, called the Loopamp 2019-nCoV Testing Reagent Kit, was launched in mid-March. In an evaluation of the kit in 76 patient nasopharyngeal samples published in the *Journal of Clinical Virology* in May, the test had sensitivity and specificity of 100 percent and 98 percent, respectively, compared to RT-qPCR.

Of course, the technology can also be adapted in other ways as well. Mason and Anders have independently used the RT-LAMP technology in sequencing workflows, for example. Mason's team uses the LAMP upfront of shotgun metagenomics sequencing while Anders and colleagues developed a multiplexed sequencing protocol. This angle is also taken by Oxford Nanopore's LamPore test, which will soon be rolled out across the UK.

Still other groups have been working on tweaks of LAMP-based testing. At the University of Pennsylvania, Haim Bau and colleagues are crafting a method called RAMP into a SARS-CoV-2 assay. The technique nests an isothermal method called multiplex recombinase polymerase amplification, or mRPA, with LAMP in a microfluidic cartridge. The team showed its colorimetric Penn-RAMP test performed better than LAMP alone for SARS-CoV-2 detection and had a sensitivity on par with RT-qPCR testing in a February *ChemRxiv* preprint.

In an email, Bau said that a few companies have approached the team expressing an interest in COVID-19 Penn-RAMP and its multiplexing capabilities. "It still remains to be seen where these interactions will lead us," he said.

Commercial tests from Color and others

The RT-LAMP chemistry is also the foundation of a handful of commercial SARS-CoV-2 test in the US and internationally.

Mason was a consultant with Color in the development of its test that uses the RT-LAMP reagents from NEB. Color obtained an EUA for a commercial lab RT-LAMP assay on May 20.

"I was very elated," Mason said. "They took something that was kind of a crazy idea at the bench in late February and early March, and then within 30 days they had the EUA written up and submitted," he said.

Color's EUA initially supported running the RT-LAMP-based testing service in a new commercial lab, but the firm has since also received a unique EUA for unmonitored, self-collected, dry nasal swabs on July 27.

With RT-LAMP, Color has also taken what has arguably been thought of as a rugged, wet lab technology for low-resource settings and made it space-age, with automated liquid- and plate-handling robots, super-dense reaction plates, and optical readers. The firm's protocol specifically uses automated high-throughput sample prep robots from Hamilton and Perkin Elmer, and Color has made its protocol available for other labs to replicate.

Now, the firm's RT-LAMP testing supports approximately three quarters of all diagnostic testing in San Francisco, a representative noted in an email.

The dry nasal swab Color uses for its sample needs a spun polyester swab and a simple tube, both of which are in abundant supply. Overall, the collection kit costs less than $1, and the samples don't require viral transport media so don't need much in the way of special shipping or handling. And, the firm claims to have found that dry nasal swabs provide a more consistent sensitivity and stability profile.

Color's core business had heretofore been population genomics testing. "We knew that addressing our national testing shortage would require a dramatically more scalable approach to lab design, which is what led us to LAMP," the representative said. "We thought that we could have a substantial impact by taking an "uncorrelated" technology bet — which is partially why we chose LAMP — and connect it to our end-to-end infrastructure, which is at the heart of our ability to deliver scaled testing that is convenient, accessible and fast."

Test volume has now more than tripled in the last month, and Color provides testing services for Marin and Alameda counties, nearly a dozen universities, including the University of Southern California, and more than 40 private employers throughout the United States.

Despite this, Color still has an average turnaround time of less than 24 hours, "and nearly all results are returned within 48 hours of reaching our lab," the representative noted.

Pricing for Color's service depends on the use case and scale but is less than Medicare reimbursement rates, Color's representative said. The self-test in particular supports "substantial savings" by reducing the need for clinical staffing.

In addition to Color, isothermal amplification methods are used in other rapid tests with US EUA, including the Abbott IDNow and the Atilla Biosystems iAMP COVID-19 assay. Abbott uses nicking enzyme amplification reaction, or NEAR, while Atilla uses a proprietary isothermal chemistry it calls OMEGA. Meridian Bioscience's Alethia platform — formerly known as Illumigene — runs a menu of LAMP-based infectious disease assays, but the firm is reportedly developing a rapid test for its PCR-based point-of-care Revogene platform instead.

Meanwhile, Branford, Connecticut-based Tangen Biosciences recently won a contract from the Biomedical Advanced Research and Development Authority (BARDA) for a point-of-

care SARS-CoV-2 assay on the firm's LAMP-based GeneSpark instrument. Tangen is also developing a combined COVID-19 and Flu A/B test, and the assays and instrument will be incorporated into a disease surveillance kit through an exclusive agreement with LabWare.

Internationally, there are a few RT-LAMP tests for SARS-CoV-2 screening commercially available as well.

Dublin, Ireland-based Hibergene began developing a LAMP-based SARS-CoV-2 test on its low-cost HG Swift instrument in February, and obtained the CE mark in May. The test is for nose and throat swab samples, and the instrument runs four assays at a time with positive results returned in 30 minutes using LAMP technology originally licensed from Eiken in 2014. Hibergene CEO Simona Esposito has previously disclosed that the firm intends to launch its system in the US next year.

Optigene also manufactures CE-marked LAMP-based COVID-19 assays that can use swab samples or extracted RNA. The tests run on each of the UK-based firm's three test instruments, and a saliva-based test was rolled out in June to the city of Southampton on the southern coast of England.

Grassroots on fire

With freely available RT-LAMP protocols and inexpensive reagents not subject to the current supply chain pinch, many groups are now getting wise to the potential benefit of RT-LAMP-based screening programs.

Historically, LAMP has been used to test for the kinds of infections that strike people in remote places with less access to clinical care, such as schistosomes or Zika, and in cases where a faster point-of-care molecular test could be of the most use. Likewise, Eiken recently launched its own diagnostic test to support malaria eradication with support from the Foundation for Innovative New Diagnostics, and the NEB colorimetric technology has been specifically applied to tests for river blindness as well as Zika.

But amid the pandemic, high-resource settings may as well be remote villages in terms of the ability to get the testing supplies they need in a timely manner.

With so many people testing out RT-LAMP, Mason founded a global LAMP R&D working group, called gLAMP. The group meets weekly using video conferencing and is officially what he calls "pre-competitive," Mason said. "We want people to work together to find out what works, and more importantly, what doesn't work, so we can learn from each other."

Rapid test development is also happening in some more unexpected places.

Among the earliest published assays, the test from Beaumont Health System was developed by urology researchers, for example.

Another member of the gLAMP working group, behavioral neurobiologist Andrés Bendesky, is crafting a COVID-19 screening test with colleagues at Columbia University's Zuckerman Mind Brain Behavior Institute.

The test is an RT-LAMP-based home test using saliva samples. "We are gearing up to use this test at Columbia to evaluate empirically how testing asymptomatic people every weekday helps prevents SARS-CoV-2 transmissions at work," Bendesky said in an email. The team is using some NEB reagents and has also developed some of its own for the test kits.

Harvard neurogeneticist Constance Cepko and her colleagues published an RT-LAMP test in *MedRxiv* in April that incorporates basic NEB LAMP reagents and novel sample prep.

Specifically, it uses glass milk, "a silica powder that is extremely inexpensive and not at all limited in supply," she said in an email.

The team picked RT-LAMP as an alternative to standard PCR because "it has a different supply chain and can be run quite cheaply, without the need for a thermocycler or a fluorescent reader." Indeed, the only equipment needed is a heating element for the isothermal reaction, which she said could be a water bath, or even a kitchen sous vide.

Similarly, a team of geneticists and neurogeneticists at Washington University in St. Louis published an extraction-free RT-LAMP assay in *MedRxiv* in May that uses the NEB colorimetric reagents and saliva samples, while a group at the Columbia University Fertility Center described their saliva-based high-performance LAMP assay, or HP-LAMP, in *MedRxiv* in June. The Columbia test has since been licensed to Sorrento Therapeutics, and the firm plans to market it under the brand name Covi-Trace.

Clinical infectious disease assay development is traditionally the domain of microbiologists and virologists in clinical labs and in industry, so it may seem like a reach for urologists, neuroscientists, and fertility specialists. But all these researchers have bench lab skills that can easily translate. Futhermore, Cepko said she and her team developed their assay "out of a desire to make a contribution to the diagnostics area," she said, particularly given the US crisis in testing.

Although Bendesky admittedly had no previous experience developing infectious disease diagnostic tests, "back in March I thought my expertise in molecular biology and genomics could be used to develop a molecular COVID test," he said. And now, with a rapid, low-cost test in hand, he said "it's been deeply heartening" to see how people with diverse skill sets were able to come together "to share ideas, results, and resources."

The need for speed

Just how sensitive do SARS-CoV-2 tests need to be? The answer depends somewhat on whether the test will be used for surveillance, screening, or diagnosis of a patient with a suspected case of COVID-19.

For screening, a preliminary study published in *MedRxiv* in June modeled the tradeoffs between test accuracy and speed, concluding that fast tests do not need to be super sensitive to make a huge impact on disease transmission.

Specifically, the epidemiological modeling incorporated variables like test sensitivity, testing frequency, and time to results. According to Daniel Larremore, lead author on the study and a computer scientist at the University of Colorado, Boulder, the modeling essentially involved the creation of a new framework, which included viral load and the way it interacts with infectiousness. This model was designed "to examine how testing can find infected individuals and get them the information they need to isolate or quarantine," he said in an email, and the resulting data was also plugged into existing epidemiology modeling frameworks to show how testing relates to viral spread at a population scale.

Anders noted that the RT-LAMP workflow is well-suited for screening. "If you want to screen all 3,000 pupils in a school, or all 10,000 employees in a workplace, then RT-LAMP can be used to process a few thousand samples with just one technician in a small lab," he said. Indeed, his group is now testing larger cohorts.

And in testing a large population, "You're not interested in who precisely has the infection — you want to know, 'Is this place clean, or is there a new hotspot coming?'" Anders said. If there is somebody infected in a school, for example, he is probably not alone, and after a day or two there will likely be more.

Furthermore, viral loads are proving to be quite high early on in COVID-19 infection.

"On a Ct scale, more people who are contagious quickly rise to a viral load of Ct 20, and then it drops by about one or two cycle thresholds per day," Anders said. In a brewing hotspot, "We have good confidence we can detect at least one or two patients in a first-level screening," and then a more sensitive test can be brought in.

The test Anders and his team developed has "a very good sensitivity for a Ct of up to 30, which corresponds to something like 1,000 molecules in the sample," he said, adding, "The best qPCR tests probably detect 100, or even 50 molecules." But patients with very active infections can have billions of viral copies, and the question, he said, remains, "With 100 viral copies, are we still even contagious?"

So, if a test is understood to detect infectivity, rather than diagnose infection, then lower sensitivities are actually not so bad.

In July, the FDA also clarified on its FAQ page that it generally does not regulate surveillance tests, but it does regulate screening and diagnostic tests. Regardless, any testing where the result will potentially be reported to patients themselves must be performed according to CLIA guidelines.

Larremore said he thinks it is possible to explain sensitivity and specificity to the general public so that people can get on board with frequent large-scale population screening, even if it is less sensitive and specific than a diagnostic test. In the end, he said, "I want to be able to take a test twice a week and know that folks in the community are doing the same."

To get to that goal, grassroots rapid test development will likely continue to scale up and be incorporated into the larger diagnostics ecosystem.

Mason leads the science team at OpenCovidScreen, a non-profit collaborating with XPRIZE on a $5 million rapid COVID testing competition. The winning contributions to the competition will be scaled up with $50 million contributed by the likes of Anthem, Amazon, Ancestry, Google, Illumina, and Thermo Fisher Scientific.

Mason said that to date almost 300 developers have signed up for the prize, and he expects to ship proficiency test kits to contestants by the first week of September.

Now that the Racine screening program is up and running, Mason is also talking to other municipalities and is looking into the potential for CLIA exemption, he said. The team has adapted the assay for use on an automated device called the Tiny Isothermal Nucleic acid quantification sYstem, or TINY, originally developed at Cornell in 2017. He plans to submit an EUA package for the test and instrument to the FDA soon.

Although it may seem like there are a ton of people driving toward the same goal with the same main ingredients, there may be quite a few assays that ultimately come to fruition and get commercialized, Mason said.

"In the gLAMP working group there are dozens of other groups thinking about ways to make [RT-LAMP] more automated and easy. I think it is a question of "how many," not "if" we'll have either CLIA-waived or automated devices that run LAMP for COVID," Mason said.

O'Conner, too, is supporting the grassroots groundwork for more RT-LAMP-based screening. "We make all of our information available online and hope others take it, improve it, remix it, and collectively we can have a grassroots effort to improve testing in communities where there are scientists with the basic molecular biology skills to perform this sort of testing," he said. His team is also working with a local company, Salus Discovery, on a point-of-care RT-LAMP test as part of the NIH RADx program, he said.

"It's important to push ferociously on all fronts, especially socializing people to the need for daily testing and understanding what the social drivers are for repeated testing," O'Connor said. "If working in HIV prevention has taught me anything, it's that the greatest solutions in the world are meaningless unless those in the community want to take advantage of them."

And, at long last, Mason said it seems that RT-LAMP is "no longer the punk upstart kid at the molecular diagnostics COVID party." The cousin of PCR stands ready to be deployed in rapidly screening many hundreds of thousands of samples per day for COVID-19, he said.

At least for now, there will likely — and unfortunately — be much more needed.