NEB EXPRESSIONS

A scientific update

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Mitigating Risk and Ensuring Consistent Supply Chain Through Internal SARS-CoV-2 Testing with RT-LAMP

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The COVID-19 pandemic posed a significant threat to the functioning of laboratories and workplaces worldwide, including here at New England Biolabs (NEB®) and our biotechnology operations in Massachusetts. As the pandemic disruptions began to take hold, it was essential to maintain our ability to produce critical materials for molecular diagnostic tests and mRNA vaccine development. Although the traditional clinical diagnostic supply chain quickly became stretched, with nasopharyngeal (NP) swabs and kits for automated RNA extraction in high demand, we were able to leverage unique resources at NEB, including >35 years of experience in infectious disease diagnostics and industry-leading expertise in simple molecular assays. As described here, we established a CLIA-certified onsite testing program utilizing saliva and colorimetric loop-mediated isothermal amplification (LAMP), and to date we have processed over 35,000 SARS-CoV-2 tests for our employees. This testing program has been an invaluable tool in keeping our workplaces and staff safe, ensuring our ability to support the global biotechnology community that relies on our reagents and products.

Our first COVID screening program utilized an outside testing provider, Color Health, who were the first to obtain an Emergency Use Authorization from the FDA for an RT-LAMP test (1). This test utilized NEB reagents and was extremely easy to use. However, it required shipping of nasal swabs to a testing lab, which occasionally resulted in delays or failures as materials were held longer than acceptable times due to weather and other unforseen circumstances. Our research groups were already evaluating the compatibility of our colorimetric LAMP technology with saliva, so we increased focus on this work through an Institutional Review Board (IRB) program with volunteer employees and family members. Sample collection and treatment options were tested and refined under the IRB and the diverse sample pool it allowed us to access. However, to enable release of test results to all individual employees and contractors, we went through the process of CLIA certification for our laboratory and test, adopting all training, operation, and documentation requirements for CLIA operation.

Why use RT-LAMP?

NEB has long been a leader in enzymes for molecular diagnostics, with isothermal amplification and LAMP of particular focus. Since its development in the early 2000s (2), LAMP has shown shown the potential to broaden access to diagnostic technology, bringing these methods outside the clinical laboratory to field and point-of-care settings. NEB has facilitated this shift by developing the first WarmStart® enzymes and colorimetric LAMP master mixes, and also by demonstrating the utility of these tools in our longstanding parasitology and neglected tropical disease research (3-5). LAMP also displays a remarkable tolerance for direct sample inputs without requiring nucleic acid purification, a critical benefit for testing outside the traditional clinical diagnostic infrastructure. With our LAMP expertise and its ability to enable rapid, simple diagnostic tests, we naturally thought of colorimetric LAMP as a new approach to SARS-CoV-2 testing.

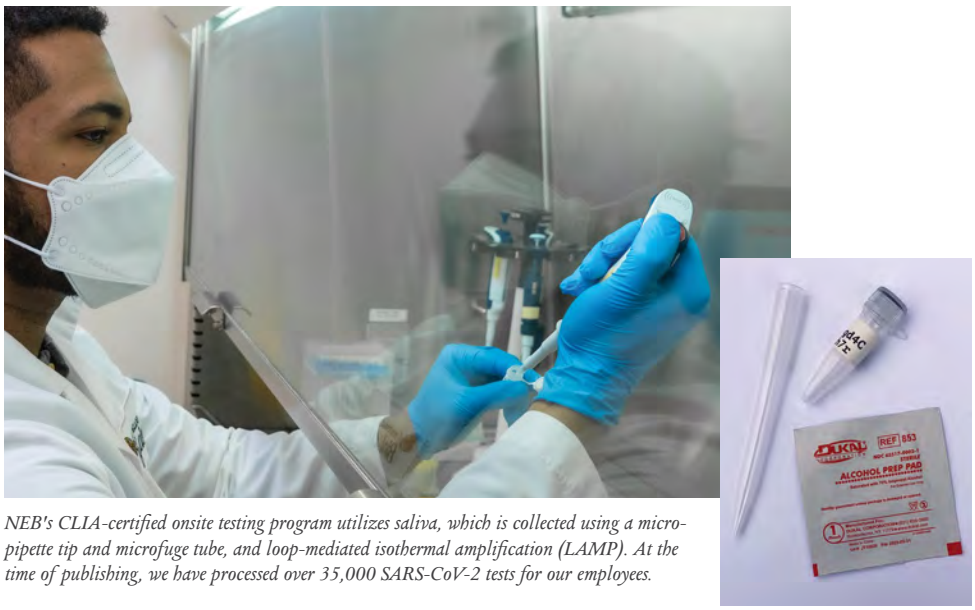
In February 2020, we quickly worked with a partner lab in Wuhan, China to demonstrate the ability of colorimetric LAMP to detect SARS-CoV-2 directly from nasopharyngeal swab samples (6). While this was a very limited and preliminary study, it demonstrated the strong potential for simplified testing. Immediately following this work, we continued optimizing the conditions and assay (7) to create a reliable colorimetric LAMP testing kit for SARS-CoV-2 (SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit, NEB #E2019), and with this product in hand, looked to utilize it for in-house testing.

Why saliva?

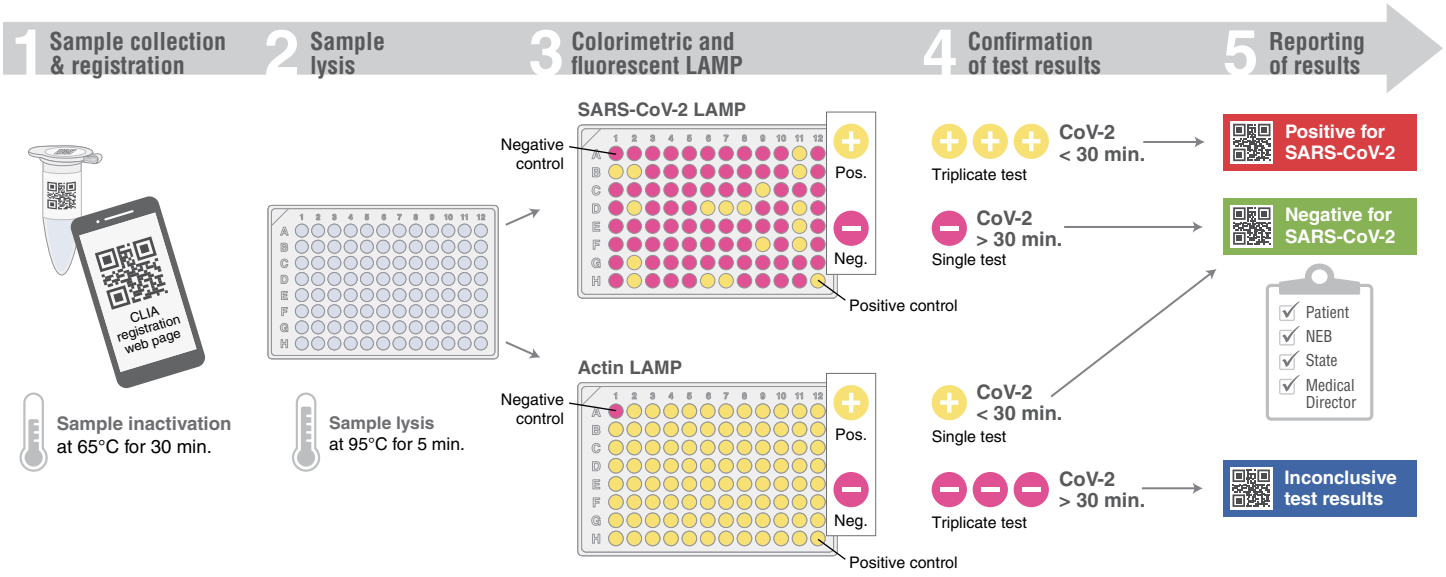
We chose to focus on saliva for our test to avoid the NP swab supply bottleneck, and also to avoid a requirement for nucleic acid extraction from the transport media sample, making the workflow as rapid as possible. Saliva collection is simple, with no swabbing required, and is preferred by our employees. Our colleagues at Yale University had pioneered the SalivaDirect workflow (8), and similar extraction-free approaches were beginning to be demonstrated for LAMP (9-10). While dedicated saliva collection devices exist, we saw no need to take those robust, transport-oriented solutions, and instead utilized a collection kit from laboratory supplies present in abundance in our facility: a 1.5 ml screw-cap tube and a 1 ml unfiltered micropipette tip for accurately and cleanly directing saliva into the tube. Employees scan QR code labels affixed to the tubes with their smartphone, enabling the tracking of samples for each registered user, and deposit their individual 1.5 ml tube into 96-well tube racks for collection.

Testing Workflow Outline

To ensure efficient sample processing with no risk to the NEB scientists conducting the tests, racks of samples are directly transferred to a 65°C incubator in our BSL2 lab for inactivation. Only



NEB's CLIA-certified onsite testing program utilizes saliva, which is collected using a micropipette tip and microfuge tube, and loop-mediated isothermal amplification (LAMP). At the time of publishing, we have processed over 35,000 SARS-CoV-2 tests for our employees.



after that step are samples handled, with equal volume saliva pipetted into a plate pre-filled with simple, colorimetric LAMP-compatible lysis buffer inspired by similar efforts (9,11). Heating samples to 95°C in this buffer serves to release RNA from cells and viral particles, as well as inactivate the accompanying nucleases that would degrade the RNA. The same QR code identifier used to log the sample is also used in the lab to record its position on the plate with a photo of the tube rack. Samples are now ready for RT-LAMP: 2 µl of each sample is loaded into a SARS-CoV-2 LAMP plate and an Actin LAMP control plate.

For a streamlined testing workflow, we turned to standard 96-well qPCR instruments (Bio-Rad® CFX96™ Touch), which are used daily by various groups here at NEB, with two of these instruments dedicated specifically to the CLIA lab. The workflow for our SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit involves processing 47 samples in one 96-well plate, with each sample tested in side-by-side reactions using either SARS-CoV-2 primers or internal control (human Actin) primers to confirm the presence of sample nucleic acid. Additionally, each plate includes one positive and one negative control. We adjusted this strategy slightly for higher throughput using the two designated instruments, with 94 samples distributed between Actin control and SARS-CoV-2 test plates, with positive and negative controls on each. Each plate is scanned using a simple flatbed document scanner before amplification to record the color of reactions, which all should begin as a bright pink color using our colorimetric LAMP mix. This is a built-in indicator of sample quality. In rare cases (<0.1% of samples) we observe an orange or yellow color after lysed saliva is added to the colorimetric LAMP mix, a sign of low pH, that may affect amplification performance. For detection of the LAMP reactions, we supplement the colorimetric mix with our LAMP Fluorescent Dye (NEB #B1700; equivalent to SYTO™-9). This is not required for measurement of LAMP because the resulting color change could easily be detected by eye or quantified using absorbance measurements, as with Color Health's test (1), but we took advantage of existing in-house LIMS software built to take results from Bio-Rad CFX files for streamlined processing and analysis. We used amplification time thresholds to call reactions as either positive (detected actin, detected SARS-CoV-2), negative (detected actin, not detected SARS-CoV-2), or inconclusive (not detected actin). When the data from a plate is released, a clinical report is automatically generated with the date and important information for using the test result. Each user with a logged sample receives a report of their result via email.

Testing Program Results

The turnaround time for one complete run of 94 samples is ~2 hours, including the sample inactivation and RT-LAMP amplification steps. On average, the user experiences a longer wait time, as sample submission is of course variable (the first tube on the rack will wait a little while for the rack to fill up). We also collect samples from our multiple Massachusetts locations, and all samples received before a designated cutoff time on testing day are reported back to the employee the same day. Any initially positive or inconclusive sample is repeated in triplicate, and only if confirmed is reported to the user to ensure accurate results.

To date (March 1, 2022) we have processed 35,461 tests from 756 individuals, with 0.49% returned positive. In 0.11% of tests, we returned an inconclusive result, and employees who receive repeated inconclusive results generally have difficulty producing sufficient saliva. These employees are advised to either change the time of day for collection or adjust the process (e.g., drinking water immediately before collection), and these remedies have resulted in a high success rate. With our ability to rapidly test and release results, positive cases can be identified and kept off premises, and any close contacts are re-tested frequently. Follow-up re-testing is performed when samples are easily attained and we can observe the dynamics of infection over time. Negative test results are required before an on-site return (typically by ~Day 8). Trends in our results have matched the general population, with very few positives observed through summer 2021, and then increasing numbers in September-December, following the Delta wave, and increasing significantly with Omicron in December 2021-January 2022. These trends allow us to adjust our workplace policies and non-pharmaceutical interventions as needed to ensure the safest possible work environment, restricting the use of meeting rooms and requiring masks in response to case numbers onsite.

With the world-class sequencing expertise and resources at NEB, we additionally can perform next generation sequencing on RNA extracted from ever positive sample, validating the performance of our sequencing products with real-world variants and sublineages, and contributing to the global scientific knowledge base of SARS-CoV-2 spread. Following with the case trends, all samples detected and sequenced in Fall 2021 were determined to be B.1.617.2 (Delta), which completely transitioned mid-December to B.1.1.529 (Omicron).

The sample position on the plate is photographed and recorded using the QR code.

Summary and Outlook

With our CLIA authorization, established workflow and equipment for testing, and general appreciation for the additional layer of workplace safety that our testing provides, we plan to continue our SARS-CoV-2 operations for the foreseeable future. And while our process works here at NEB, we continually advise others on the robustness and flexibility of RT-LAMP to accommodate other testing plans at a wide range of scales and settings. Examples include a similar workflow at Rice University (12), screening healthcare workers in Nicaragua (13), and an effort across multiple African countries spearheaded by ICGEB (14). Platforms as simple as heat and visual detection can be used for low-throughput settings (15), small portable instruments for fieldable moderate throughput needs (16), and higher complexity workflows for high-throughput testing programs (17-18). With the experience and testing infrastructure we have built, setting up a test for potential new viral threats will be achievable with rapid timelines, and we will consider influenza or other target tests as needs arise. The ability to provide routine diagnostic testing has helped us safely continue our research and production operations through the ongoing pandemic, and we feel more environments and congregate settings adopting initiatives like ours would significantly benefit public health and viral control efforts.

For more information, read our preprint: **'Development and Implementation of a Simple and Rapid Extraction-Free Saliva SARS-CoV-2 RT-LAMP Workflow for Workplace Surveillance'** at https://www.medrxiv.org/content/10.1101/2022.03.11.22272282v1

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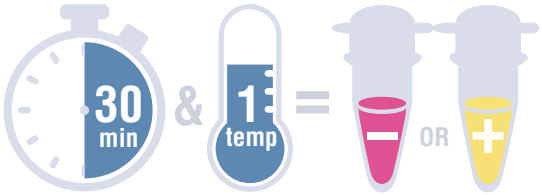
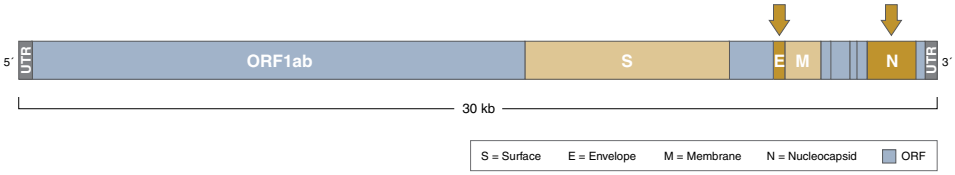
FEATURED PRODUCT

SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit

The SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit utilizes loop-mediated isothermal amplification for use in the analysis of SARS-CoV-2, the novel coronavirus that causes COVID-19.



The kit includes WarmStart Colorimetric LAMP 2X Master Mix with UDG and a primer mix targeting the N and E regions of the viral genome. Controls are provided to verify assay performance and include an internal control primer set and a positive control template. Guanidine hydrochloride has been shown to increase the speed and sensitivity of the RT-LAMP reaction and is also included.



SARS-CoV-2 genome. Arrows indicate regions targeted by assay.

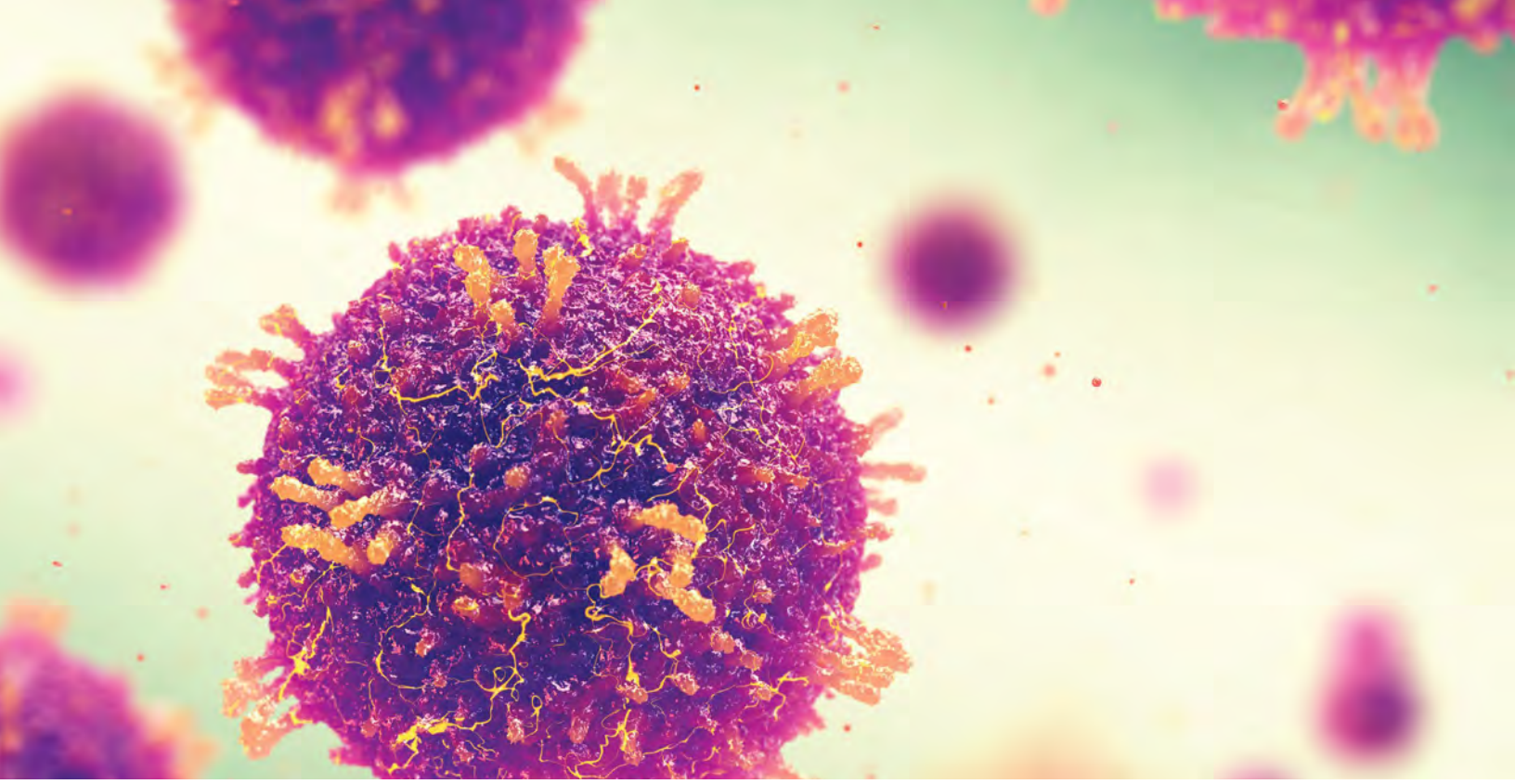
Learn more at
www.neb.com/E2019

Highlights

- Colorimetric LAMP enables simple, visual detection (pink-to-yellow) of amplification of SARS-CoV-2 nucleic acid
- Set up reactions quickly and easily, using a simple heat source and unique WarmStart® technology
- Reduce risk of carryover contamination, with UDG and dUTP included in the master mix
- Assay targets N and E regions of the SARS-CoV-2 genome for optimized sensitivity and specificity
- Bring confidence to your results using the provided controls

Ordering information:

Product	NEB #	Size
SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit	E2019S	96 rxns
Component sold separately: WarmStart Colorimetric LAMP 2X Master Mix with UDG	M1804S/L	100/500 ml



It's time to think differently.

Find out how NEB can support your infectious disease research and development.

Gaining a better understanding of infectious diseases, including their characterization, evolution and transmission, continues to be a priority, both from an R&D standpoint and as a public health issue. The COVID-19 pandemic has demonstrated the need for a wide range of tools to research infectious diseases, and has highlighted the importance of speed and the ability to pivot as new problems arise. This has emphasized the need for innovation and thinking differently about where to access those critical materials, including genomics reagents.

Many scientists know NEB as a trusted reagent supplier to the life science community, but what you may not know is that we also offer a portfolio of products that can be used in infectious disease research, development of diagnostics and therapeutics, and in epidemiological studies and disease surveillance. In fact, many of our products have supported the development of COVID-19 diagnostics and vaccines, and can also be utilized with other infectious diseases, such as influenza and malaria.

- Benefit from almost 50 years of experience in molecular biology & enzymology
- Partner with our OEM & Customized Solutions team to find the best solution for your needs
- Take advantage of our expanded manufacturing capabilities
- Access product formats, such as GMP-grade*, lyophilized, lyo-ready and glycerol-free
- Be confident in your product performance with our expanded quality and regulatory systems

Ready to get started? Learn more at
www.neb.com/InfectiousDiseases

** "GMP-grade" is a branding term NEB uses to describe reagents manufactured at our Rowley, MA facility, where we utilize procedures and process controls to manufacture reagents under more rigorous conditions to achieve more stringent product specifications, and in compliance with ISO 9001 and ISO 13485 quality management system standards. NEB does not manufacture or sell products known as Active Pharmaceutical Ingredients (APIs), nor do we manufacture products in compliance with all of the Current Good Manufacturing Practice regulations.*

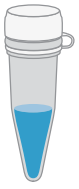
NEW PRODUCT

It's prime time!

Introducing LyoPrime Luna™ Probe One-Step RT-qPCR Mix with UDG

Supplied as a lyophilized cake, the LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG enables sensitive detection of target RNA sequences in a room temperature-stable format. This product contains the same versatile features and strong performance as the liquid version: Luna® Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019).

LyoPrime Luna offers a robust, versatile RT-qPCR option in a shelf-stable lyophilized format

	Luna® Probe One-Step RT-qPCR 4X Mix with UDG	LyoPrime Luna™ Probe One-Step RT-qPCR Mix with UDG
	NEB #M3019	NEB #L4001
	4X Master Mix	Lyophilized Master Mix
		
Number of tubes	1	1
Sample-limiting concentration	4X	2–4X
dUTP included	✓	✓
UDG included	✓	✓
Universal ROX included	✓	✓
Storage temperature	–20°C	Room temperature

The LyoPrime Luna RT-qPCR Mix (NEB #L4001) offers the same versatile features as the Luna RT-qPCR 4X Mix (NEB #M3019) in a lyophilized format that can be shipped and stored at room temperature.

Advantages:

- Simply add nuclease-free water for rapid rehydration
- Store at room temperature for up to 2 years prior to rehydration
- Eliminate cold chain shipping requirements
- Multiplex up to 5 targets to increase throughput
- Increase reaction specificity and robustness with our unique pairing of Luna WarmStart® RT and Hot Start *Taq*
- Prevent carryover contamination with Thermolabile UDG and dUTP, included in an optimized mix
- Maintain RNA integrity with Murine RNase Inhibitor, included in an optimized mix
- Eliminate pipetting errors with non-fluorescent blue tracking dye
- Use with a variety of instrument platforms, including those that require a high or low ROX reference signal
- Developed in collaboration with Fluorogenics™ Limited, a wholly owned subsidiary of New England Biolabs, Inc.®

Ordering information:

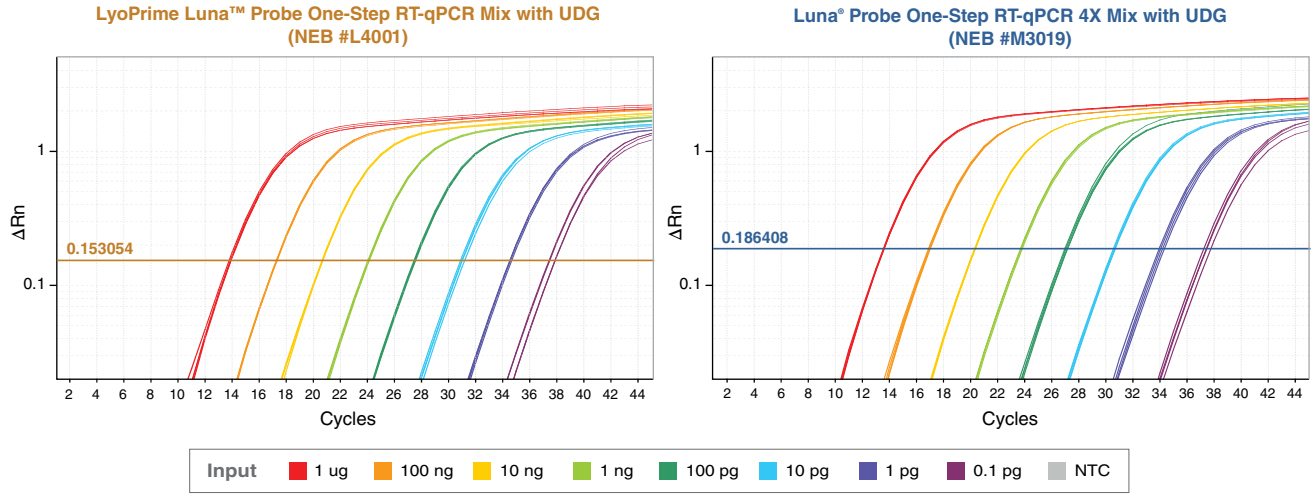
Product	NEB #	Size
LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG	L4001S	120 rxns
Companion product: Nuclease-free Water	B1500S/L	25/100 ml

Interested in learning more about whether lyophilization is the right product format for you?

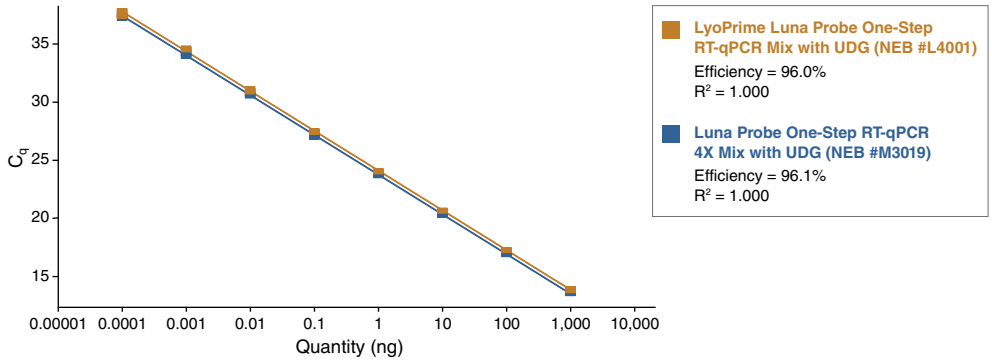
Download our whitepaper on technical considerations for lyophilization at www.neb.com/Lyophilized_reagent_development

Lyophilized and liquid Luna RT-qPCR mixes demonstrate equivalent strong performance

A. Amplification plot comparison

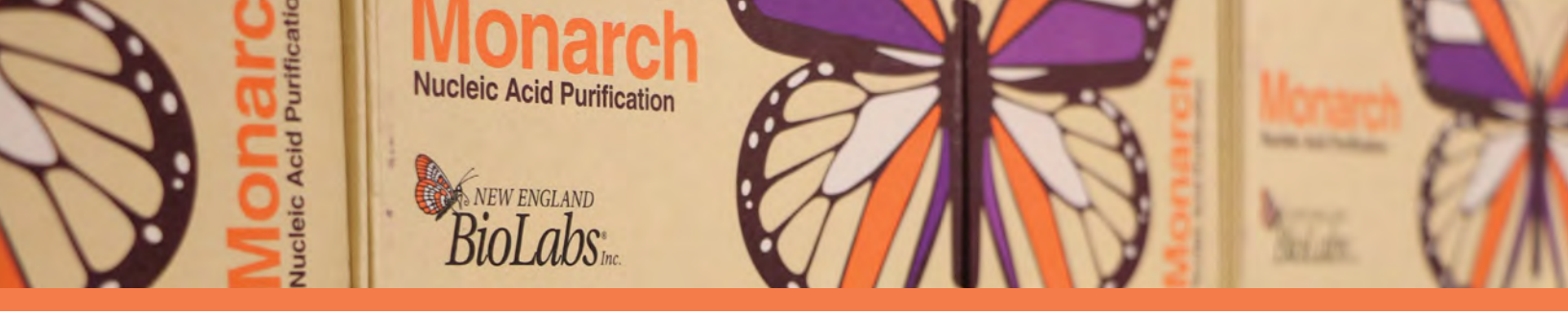


B. Standard curves (overlay)



A. RT-qPCR targeting human β-actin was performed using either the LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001) or Luna Probe One-Step 4X Mix with UDG (NEB #M3019) over an 8-log range of input template concentrations (1 µg – 0.1 pg Jurkat total RNA) with 4 replicates at each concentration, run on an ABI QuantStudio® 6 Flex real-time instrument. Reactions (20 µl) included primers at 400 nM each and and probe at 200 nM, and followed the product recommended cycling conditions.

B. Standard Curve results were substantially equivalent for the lyophilized (gold) and liquid-format (blue) mixes, with strong linearity and reproducibility observed, even at the lowest concentrations tested.



Monarch® DNA & RNA Purification Kits – Designed with sustainability in mind

At NEB, we continuously strive to promote ecologically sound practices and environmental sustainability in order to protect our natural resources, both locally and globally. For over 5 years, we have beeen designing our Monarch DNA & RNA purification kits & products with sustainability in mind by purposefully reducing plastics and packaging without sacrificing performance & quality.

Reduced lab waste

Significantly less plastic as compared to leading supplier
Monarch kits still deliver high yields, purity and performance

Thinner-walled columns
Reduction in total plastic without affecting performance

Buffer bottles
Carefully designed to minimize plastic usage

Flexible purchasing options

Buffers and columns sold separately
Purchase only what you need and avoid wasted materials

Same performance, design and formulations
Standalone products are the same components that are included in complete kits

MONARCH®
Sustainability

No excessive packaging

Sturdy, reusable boxes at just the right size
Carefully designed to eliminate empty space, versatile Monarch boxes can be reused anywhere

Concise protocol cards replace printed manuals
Both cards and manuals are available online as PDFs

Sustainable & recyclable packaging

Sourced for recyclability
All components are purposefully sourced for recyclability

Instructions for recycling kit components
Can be found on product packing or online

Recycled paper
Used to make the kit boxes, inserts and paper materials

Eco-friendly printing
Printing of boxes and packaging powered by green sustainable sources such as wind

We hope that others in the scientific community join us in our efforts to make nucleic acid purification greener, one step at a time.

To learn more and request your Monarch sample, visit **NEBMonarch.com**.

Bringing the International Space Station into the molecular age

by Joanne Gibson , Ph.D., New England Biolabs, Inc.

This article is an excerpt from an NEBinspired blog post, published March 10, 2022 (**www.neb.com/nebinspired-blog/bringing-the-international-space-station-into-the-molecular-age**).

The International Space Station is teeming with bacteria!

Or is it?

"It's like a dirty gym", says one media source, "It is riddled with germs", says another.

Not so, says Dr. Sarah Wallace – and if anyone knows, she does; Dr. Wallace is a lead in the microbiology lab at NASA's Johnson Space Center. Her lab is responsible for pre- and in-flight monitoring of the microbes that may be hitching a ride to the International Space Station (ISS) on cargo, in water systems, or, more commonly, on the astronauts themselves. The primary goal of Dr. Wallace's lab is to identify and quantify medically significant pathogens and assess the risk they pose to the crew.

Astronauts from 15 nations have continually inhabited the ISS since November 2000. In other words, in the past 20 years, there has never been a time when there have not been astronauts – and the millions of microbes that make up the human microbiome – on the space station. With that in mind, you might be surprised to hear that culture-based analysis of the first 29 missions to the ISS showed that it was, in fact, cleaner than the average home!

NASA has its sights set on exploration further into space than the ISS and low-Earth orbit, namely back to the Moon and eventually to Mars. Unlike the ISS, the spacecraft involved in these missions will not be regularly replenished with supplies or have the ability to return samples to Earth for analysis. Therefore, Dr. Wallace's team also creates modified-for-spaceflight molecular workflows to diagnose potential microbial-based threats in near real-time, entirely onboard the spacecraft.

Traditional microbial monitoring on the ISS relies on Earth-bound methods

In the past, the tiniest inhabitants on the ISS, both commensal microorganisms and potential pathogens, have been monitored and identified using culture techniques. However, samples collected by the astronauts onboard the ISS need to be transported back to Earth for a complete biochemical and sequence analysis and identification. Additionally, the growth media and conditions used onboard the ISS are favorable to certain microbes, while others that may be present will not grow under these conditions. Therefore, it's a little biased.

In recent years, Dr. Wallace and her team have been moving microbial identification on the ISS into the molecular age utilizing techniques such as PCR and nucleic acid sequencing.

Why monitor for microorganisms in space?

It may seem like an obvious question when posed to a scientific audience, but there are a few reasons, including one or two that you might not have considered:

1. **Diagnostic potential and environmental monitoring** – Establishing a molecular identification process would provide the space program with the ability to identify a potential pathogen in near real-time, which can guide treatment or remediation.
2. **Planetary protection** – If astronauts can characterize the microbiome of the spacecraft, the potential for contaminating the surface of the Moon or Mars would decrease significantly.
3. **Applications on Earth** – While PCR and nucleic acid sequencing are not new technologies, their application in space requires extensive methodology development, which lends itself to other applications on Earth, such as point-of-care locations.
4. **Monitoring change** – the spaceflight environment has a variety of impacts on living organisms. Characterizing phenotypic and molecular changes helps us understand the impact of the space environment on microbial behavior.

Christina Koch demonstrates pipetting onboard the ISS as she extracts and purifies DNA for downstream nanopore sequencing. The maintenance work area (MWA) serves as the astronauts' lab bench onboard the ISS.

What does molecular biology in space look like?

A palm-sized thermocycler (miniPCR®) and a small nucleic acid sequencing device (MinION®) were the foundation for a portable, remote amplification and sequencing protocol; however, deploying it on station required a gradual workflow expansion. A workflow that may appear straightforward to a molecular biologist in a lab on Earth involves many considerations concerning crew safety, environmental control, and life support systems when conducted in space. For example, water onboard the ISS is recycled, and therefore alcohol, typically used for washing nucleic acids, cannot be used in the sample prep. Additionally, lab instruments must have critical value due to the extremely limited physical space.

The ultimate goal was for crew members to have the ability to swab a surface and undertake an entire workflow through to sequencing, all while onboard, and this took years of methodology development.

What microorganisms are found on the ISS?

Because of the biases mentioned above with the culture method, microorganism identification on the ISS plateaus. Typically, organisms identified are those found in the Earth environment, such as *Staphylococcus, Penicillium,* and other microorganisms associated with human-occupied spaces. Using the MinION, a broader range of organisms are identified in addition to what is observed growing on culture media - more Gram-negative and anaerobic organisms that exist in the human microbiome are identified, giving a more authentic picture of the human biome in space.

What are the unknowns as we venture further?

When planning for a trip where samples cannot be returned to Earth for analysis, there are plenty of questions that Dr. Wallace and her team ponder:

1. **Power source** – can they plug in instruments and have the same type of power they have on the ISS? Lyophilized/ stable reagents will be vital if there is no refrigeration.
2. **Mass and volume** – everything that gets packed on the vehicles will have to have critical value. What can be miniaturized?
3. **Radiation environment** – the radiation levels in orbit around the moon will differ from the levels on the ISS. Will this affect the molecular diagnostic equipment and experiments?
4. **Dust** – yes, you read correctly…. dust. Moon dust is extremely fine and can cover the astronauts' extravehicular activity (EVA) suits - will this affect the hardware?

Dr. Wallace's team finds that the unknowns of exploration beyond low-Earth orbit provide the challenge and excitement to continually drive the planning team to find answers. And, we can't wait to follow their progress.

Updated NEBcutter® V3.0

NEBcutter was originally designed to assist users with restriction digests and traditional molecular cloning workflows, and quickly became one of the most popular online tools available at neb.com. NEBcutter is used to:

- Find restriction enzyme sites within a DNA sequence
- Visualize a virtual restriction digest on a gel to compare against results at the bench
- Determine whether DNA methylation will impact your restriction digest

We are pleased to announce the launch of the latest version of this popular tool! NEBcutter V3.0 offers an improved user interface, and includes the following features:

Download results as a PDF

Filter by types of REs (i.e., blunt, 8-base cutter, etc)

Add multiple enzymes at one time

Log in to save your projects and view from various browsers

Visualize digestion on a virtual gel

Quickly find open reading frames with our enhanced ORF Finder

Visit **NEBcutter.neb.com** to get started, and check out these online tutorials for help with some common NEBcutter applications.

- **How to determine methylation effects on restriction digests**
- **Determine the restriction enzymes that cut your DNA**
- **Visualize a restriction digest on a virtual gel**
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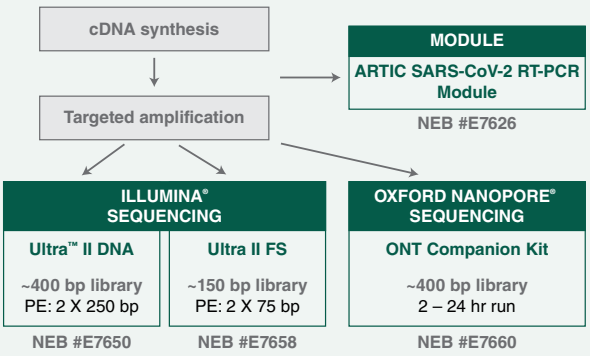


Bringing more COVID-19 testing to Africa

by Lydia Morrison, M.S. & Tessa DeVoe, New England Biolabs, Inc.

With so few Africans vaccinated against COVID-19, simple and sensitive tests are critical for saving lives. NEB is donating reagents to aid in the development of diagnostic tests (cLAMP) that can be used instead of PCR and can deliver rapid results detected by a simple color change. They are ideally suited for resource-poor areas by not requiring expensive laboratory equipment.

The state of pandemic efforts in Africa

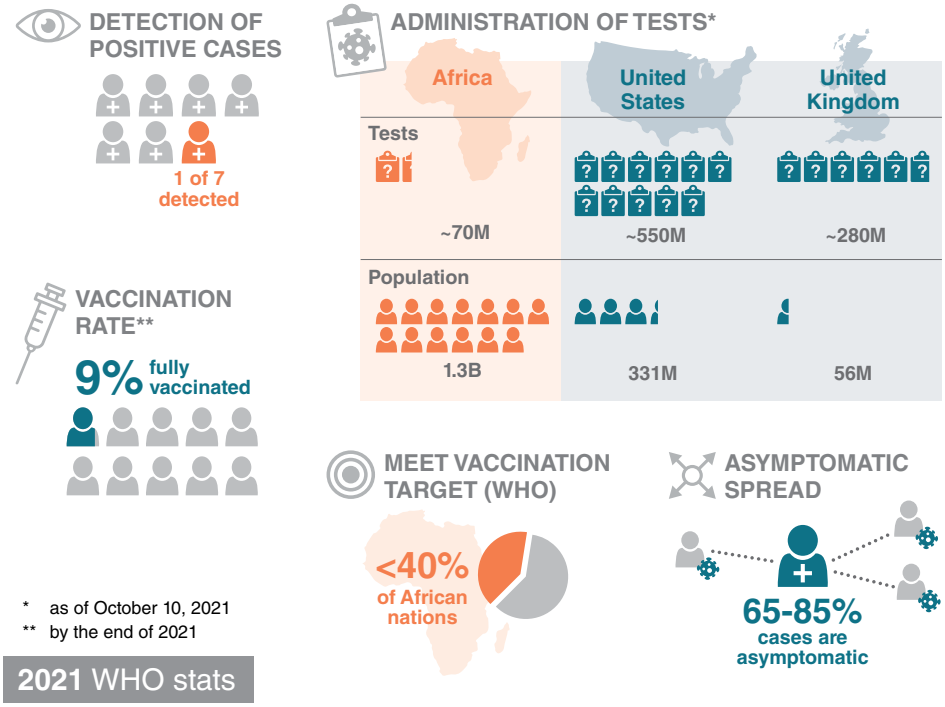
COVID-19 testing is a major challenge in Africa. In October of 2021, the World Health Organization (WHO) released an assessment of testing in Africa, in which it estimated that 6 out of 7 COVID-19 infections go undetected. By October 10th of 2021, Africa had administered about 70 million tests to its population of 1.3 billion. For a point of reference, the U.S. had administered over 550 million tests to its population of 331 million, and the United Kingdom had administered 280 million tests to its population of 56 million.

Vaccination rates also lag with most African nations failing to reach the 40% vaccination target set by the WHO by the end of 2021. Only about 9% of Africans were fully vaccinated by the end of 2021.

Without higher vaccination rates, increased testing can be used to protect communities from high rates of viral spread. Estimates indicate that 65% – 85% of the cases are asymptomatic, making it easy for infected individuals to spread the virus without their knowledge. To put it bluntly, the lack of inexpensive COVID-19 testing is a big problem in Africa.

African labs are successfully piloting RT-LAMP for molecular diagnostics

Efforts are being made to address the shortcomings of the COVID testing system in Africa. The WHO Regional Office for Africa announced in October 2021 that it would focus $1.8 million in 8 countries to enhance COVID-19 community screening and testing accessibility, aiming to reach some 7 million Africans. The program employs a strategic approach to contact tracing, and provides rapid antigen diagnostic testing to those exposed. It also provides hygiene kits that include face masks and hand sanitizers, to households within a 100-meter radius of an infected person.



New England Biolabs (NEB) has collaborated with the International Centre for Genetic Engineering and Biotechnology (ICGEB) to help create an alternative method to diagnose COVID-19 based on the use of the reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) assay. RT-LAMP is a rapid isothermal amplification technology requiring minimal equipment to carry out, making it an ideal method for locations with limited testing infrastructure. A multicenter observational pilot study has been completed in the African countries of Cameroon, Ethiopia, Kenya, and Nigeria, which demonstrated high specificity and sensitivity of a colorimetric RT-LAMP assay for COVID-19 detection. An expanded Phase II program will soon begin, with reagents donated by NEB, and funding by the Bill & Melinda Gates Foundation.

Test results with a simple change in color

The history of RT-LAMP development at NEB began in the early 2000s. Viral outbreaks in developing nations, such as SARS-CoV-1 (2002-2004), avian H1N1 influenza (2009), Zika (2007-2015) and Ebola (2013-2016 and 2018-2020) highlighted a need for simple, sensitive diagnostic tests that could be performed in low resource settings. With this in mind, NEB initiated research into a novel isothermal amplification technology called LAMP, which was created by Eiken® Chemical Co. Ltd.

In 2012, NEB demonstrated that the presence of a nucleic acid target could be detected using colorimetric LAMP (cLAMP) – an assay in which detection occurs through the visual color change of a dye caused by a change in pH. This detection method does not require expensive equipment. Additionally, results can be obtained in 30 minutes and its sensitivity is close to that of qPCR. More recently, NEB was able to show that RT-LAMP provided sensitivity similar to RT-qPCR in the detection of COVID-19 from saliva rather than nasal swabs, making sample collection less invasive.

Overall, continued efforts are needed to enhance testing accessibility within African countries. Africa represents an important part of the global community and access to testing and vaccines impacts the state of the COVID-19 pandemic around the world. The success of alternative low-cost, rapid diagnostic methods in Africa could be a blueprint for other nations struggling to meet their population's COVID-19 testing needs.

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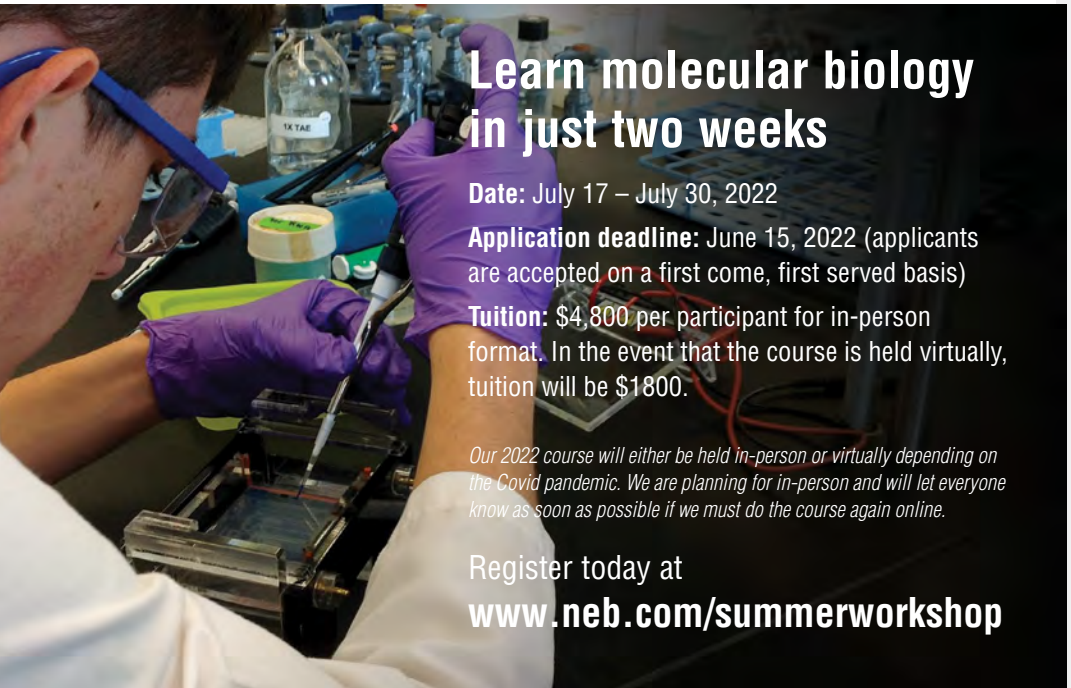

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