JOURNAL OF BIOMOLECULAR TECHNIQUES

Volume 32 (3); 2021 September

Loop-Mediated Isothermal Amplification (LAMP) Detection of SARS-CoV-2 and Myriad Other Applications

Authors: Keith J. M. Moore[49,*], Jeremy Cahill[48,*], Guy Aidelberg[4,5], Rachel Aronoff[5,6,7] Ali Bektaş[8], Daniela Bezdan[10,11,12,13], Daniel J. Butler[1,2], Sridar V. Chittur[16], Martin Codyre[17], Fernan Federici[19], Nathan A. Tanner[29], Scott W. Tighe[43], Randy True[44], Sarah B. Ware[5,45,46], Anne L. Wyllie[47] Evan E. Afshin[1,2,3], Andres Bendesky[9], Connie B. Chang[14,15], Richard II Dela Rosa[49], Eran Elhaik[18], David Erickson[51], Andrew S. Goldsborough[20], George Grills[48], Kathrin Hadasch[4,21,22,23], Andrew Hayden[16], Seong-Young Her[24], Julie A. Karl[25], Chang Hee Kim[26], Alison J. Kriegel[52], Thomas Kunstman[27], Zeph Landau[28], Kevin Land[40], Bradley W. Langhorst[29], Ariel B. Lindner[4], Benjamin E. Mayer[21,22], Lee A. McLaughlin[30], Matthew T. McLaughlin[25], Jenny Molloy[31], Christopher Mozsary[1,2], Jerry L. Nadler[32], Melinee D'Silva[32], David Ng[33], David H. O'Connor[25], Jerry E. Ongerth[34], Olayinka Osuolale[35], Ana Pinharanda[36], Dennis Plenker[37], Ravi Ranjan[38], Michael Rosbash[39], Assaf Rotem[40], Jacob Segarra[41], Stephan Schürer[48], Scott Sherrill-Mix[42], Helena Solo-Gabriele[48], Shaina To[49], Merly C. Vogt[36], Albert D. Yu[39], The gLAMP Consortium, **Christopher E. Mason[1,2,3,50,#]**

Affiliations

1. Department of Physiology and Biophysics, Weill Cornell Medicine, New York, NY, USA
2. The HRH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, Weill Cornell Medicine, New York, NY, USA
3. The WorldQuant Initiative for Quantitative Prediction, Weill Cornell Medicine, New York, NY, USA
4. Université de Paris, INSERM U1284, Center for Research and Interdisciplinarity (CRI), F-75006 Paris, France
5. Just One Giant Lab, Centre de Recherches Interdisciplinaires (CRI), Paris, France
6. Action for Genomic integrity through Research! (AGiR!), Lausanne, Switzerland
7. Association Hackuarium, Lausanne, Switzerland
8. Oakland Genomics Center, CA
9. Department of Ecology, Evolution and Environmental Biology, Columbia University, New York, NY, USA
10. Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany.
11. NGS Competence Center Tübingen (NCCT), University of Tübingen, Tübingen, Germany

12. Poppy Health, Inc., San Francisco, USA
13. Institute of Medical Virology and Epidemiology of Viral Diseases, University Hospital, Tubingen, Germany
14. Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA
15. Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
16. Center for Functional Genomics, Department of Biomedical Sciences, School of Public Health, University at Albany, State University of New York, Rensselaer, NY, USA
17. GiantLeap Biotechnology Ltd
18. Department of Biology, Lund University, Sölvegatan 35, Lund, Sweden
19. ANID – Millennium Science Initiative Program- Millennium Institute for Integrative Biology (iBio), Institute for Biological and Medical Engineering, Schools of Engineering, Biology and Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile
20. RNAssist Ltd, Cambridge UK.
21. Department of Biology, Membrane Biophysics, Technische Universität Darmstadt, Darmstadt, Germany
22. Lab³ e.V. - Labspace Darmstadt, Darmstadt, Germany
23. IANUS Verein für Friedensorientierte Technikgestaltung e.V. Darmstadt, Germany
24. Metamer Labs, Boston, MA, USA
25. Department of Pathology and Laboratory Medicine, School of Medicine and Public Health, University of Wisconsin-Madison, WI, USA
26. GoDx, Inc., Madison, WI, USA
27. University of Colorado Boulder, CO, USA
28. Dept. of Computer Science, University of California, Berkeley, CA, USA
29. New England Biolabs, Ipswich, MA
30. IC Mobile Lab Ltd
31. Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge, UK
32. Department of Pharmacology, New York Medical College, Valhalla, NY, USA
33. Zuckerman Mind Brain Behavior Institute, Columbia University, New York, NY, USA
34. University of Wollongong, Environmental Engineering
35. Applied Environmental Metagenomics and Infectious Diseases Research (AEMIDR), Department of Biological Sciences, Elizade University, Ilara Mokin, Nigeria
36. Department of Biological Sciences, Columbia University, New York, NY, USA
37. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA
38. Genomics Resource Laboratory, Institute for Applied Life Sciences, University of Massachusetts Amherst, MA, USA
39. Howard Hughes Medical Institute and Department of Biology, Brandeis University, Waltham, MA, USA
40. School of Engineering and Applied Sciences, Harvard University, MA, USA
41. Metamer Labs, Boston, MA
42. Department of Microbiology, University of Pennsylvania, Philadelphia, PA, USA
43. University of Vermont, Burlington, VT, USA
44. FloodLAMP Biotechnologies, San Carlos, CA, USA

45. BioBlaze Community Bio Lab, 1800 W. Hawthorne Ln., Suite J-1, West Chicago, IL 60185
46. Blossom Bio Lab, 1800 W. Hawthorne Ln., Suite K-2, West Chicago, IL 60185
47. Department of Epidemiology of Microbial Diseases, Yale School of Public Health, CT 06510
48. University of Florida, Miami, FL, 33146
49. School of Science and Engineering, Ateneo de Manila University, Manila, Philippines
50. The Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY, USA
51. Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, 14850
52. Medical College of Wisconsin, Milwaukee, WI, 53226
*First Authors
Corresponding author

Abstract

As the 2nd year of the COVID-19 pandemic begins, it remains clear that a massive increase in the ability to test for SARS-CoV-2 infections in a myriad of settings is critical to control the pandemic and to prepare for future outbreaks. The current gold standard for molecular diagnostics is the polymerase chain reaction (PCR), but the extraordinary and unmet demand for testing in a variety of environments means that both complementary and supplementary testing solutions are still needed. This review highlights the role that loop-mediated isothermal amplification (LAMP) has had in filling this global testing need, providing a faster and easier means of testing, and what it can do for future applications, pathogens, and to prepare for future outbreaks.

The review lays out the current state of the art for research of LAMP-based SARS-CoV-2 testing, as well as its implications for other pathogens and testing. The authors represent the global LAMP (gLAMP) Consortium - an international research collective that has regularly met to share their experiences on LAMP deployment and best practices; sections are devoted to all aspects of LAMP testing, including preanalytical sample processing, target amplification and amplicon detection, then the hardware and software required for deployment, and finally a summary of the current regulatory landscape. Included as well are a series of first-person accounts of LAMP method development and deployment. The final conclusions and recommendations section provides the reader with a distillation of the most validated testing methods and their paths to implementation.

The review aims to provide practical information and insight for a range of audiences: for a research audience to help accelerate research through sharing of best practices, for an implementation audience to help get testing up and running quickly, and for public health, clinical, and policy audience to help convey the breadth of impact that LAMP methods have to offer.

Introduction

The need to expand molecular testing options beyond that which PCR can cost-effectively deliver has been put into sharp focus by the COVID-19 pandemic. Resource-poor and resource-rich countries alike need to be able to track the virus in real-time to mitigate its spread. Here, we show how LAMP-based testing solutions have become a major part of that expansion and highlight other applications. This includes cost-effective *expansion-by-volume* (more tests per day), *expansion-by-location* (more, smaller testing centers to expand the geographical reach of testing) and bringing the test *closer to the patient* (point-of-care and home testing). The collection of LAMP methods presented here provide a toolbox to construct testing solutions for most real-world applications. LAMP is by no means the only testing method to augment PCR, but its relative ease of implementation, lower cost, and simple equipment requirements mean it can be broadly, quickly, and cheaply established.

No method is without its flaws; reverse transcription and LAMP (RT-LAMP) is certainly no exception and valuable scientific work continues to improve specificity, sensitivity, scalability, and usability. Yet, a tipping point has been reached: progress over the last year has led to the development of many useful variations on a core SARS-CoV-2 RT-LAMP assay; wide-scale LAMP-based testing has moved from being aspirational to being deployed at state and national levels. Multiple tests have been approved by regulators (**Table S1**), including a colorimetric LAMP test being approved for home use (Lucira Health) and many more under review. Here, we provide a methodologically useful guide for developing and deploying a LAMP-based test for COVID-19 and more broadly to bring us closer to a world of democratized diagnostics where everyone can benefit from advances in modern genomics to address public health challenges.

02.01 Tour of the Review

Our emphasis is primarily methodological and although focused on LAMP, many aspects are applicable to other isothermal methods (Piepenburg et al. 2006; Li et al. 2018; Niemz, 2011, Yu et al. 2021 ; Eftekhari et al. 2021 ; Chaouch, 2021 ; Tran et al. 2021). The review provides readers new to the field with an ***Introduction*** to SARS-CoV-2 testing and the underlying isothermal technology, the ***Preanalytical sample processing***, ***Target Amplification*** *and* ***Amplicon Detection*** while considering the ***Infrastructure*** required for deployment in the current ***Regulatory*** *landscape*. The ***Discussion*** considers the current state of the art and future directions, methodologically and from a public health perspective. The main text is augmented with four **Topic Boxes A-D** that address specialist topics in greater depth.

We have highlighted the different lenses and perspectives through which researchers can view LAMP technology, depending on whether they provide one-to-one patient care in a remote rural environment or conversely frequent mass testing for surveillance and epidemiology in a large public health laboratory. The series of choices (and corresponding compromises) that one makes

to create a test that is fit for a specific purpose is a common theme: regardless of one's setting, budget or scale of testing, '*there is a LAMP for that'* (**Figure 1**).

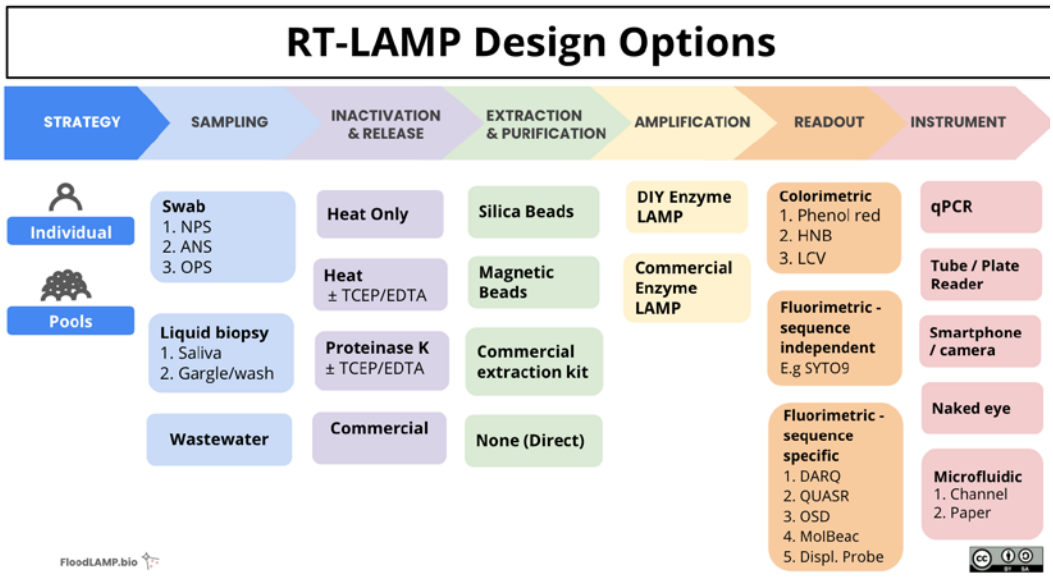


Figure 1. "We have a LAMP for that": major design choices when developing RT-LAMP tests. *At each stage in the design process, a series of decisions impact the final configuration of the test, be that for an individual patient or for surveillance testing with pooled screening. The inherent flexibility and comparative simplicity of LAMP means that for almost all settings and use cases there is one configuration of the LAMP toolbox that is fit-for-purpose. LAMP tests for use in any two settings or geographies can be dramatically different. Nasopharngeal Swab (NPS), Anterior Nares Swab (ANS), oralpharyngeal swab (OPS), tris(2-carboxyethyl) phosphine (TCEP), Ethylenediamine tetraacetic acid (EDTA), Do-it-yourself (DIY), Hydroxy naphthol blue (HNB), leuco crystal violet (LCV), Dark Quenching technique (DARQ), Quenching of Unincorporated Amplification Signal Reporters (QUASR), oligonucleotide strand exchange (OSD), Molecular Beacons (MolBeac).*

COVID-19 Testing

The emergence of a cluster of acute respiratory syndrome cases in Wuhan City, China drew worldwide attention in January 2020, with rapid epidemiological investigation identifying a novel coronavirus, SARS-CoV-2 as the causative agent of coronavirus disease 2019 (COVID-19). Once the viral genomic RNA sequence was released, the global scientific community began myriad investigations with a particular focus on diagnostic testing and vaccine development. The potential for global spread was clear and the ability to rapidly and accurately detect SARS-CoV-2 became paramount to tracing infection and controlling what quickly became a global pandemic. Researchers from Charité Berlin reported a quantitative reverse transcription polymerase chain reaction (RT-qPCR) test for the novel coronavirus in January with the protocol and primers being endorsed by the World Health Organization; the United States Centers for Disease Control and Prevention (CDC) followed suit with its own PCR test and began limited distribution in early February 2020 (Abraham et al. 2020).

The rapid production of these tests and the ability to begin testing in diagnostic laboratories was an impressive and valuable achievement. However, limited access to these tests - coupled with an overwhelming demand by healthcare systems overwhelmed by cases of COVID-19 - it became clear that relying only on traditional clinical testing infrastructures would be insufficient to track and contain the growing public health threat from SARS-CoV-2. Antigen and antibody test development began in earnest for broadening surveillance capabilities, but the need for alternative sensitive molecular methods remained. PCR tests have long been the reference method for molecular diagnostics, but the typical test workflow presents limitations to accessible, wide-scale testing. Specifically, a conventional workflow has significant requirements for nucleic acid extraction from samples, sophisticated and expensive real-time fluorescence thermocyclers, and trained personnel in certified Biosafety Level 2 (BSL2) or BSL3 testing laboratories.

02.03 Loop-Mediated Isothermal Amplification (LAMP)

Nucleic acid amplification techniques that avoid such constraints and hold promise as a companion method to RT-qPCR testing have been developed. Notable amongst these is reverse transcription loop-mediated isothermal amplification (RT-LAMP). First described in 2000 (Notomi 2000), LAMP utilizes six target-specific primers for highly specific and fast amplification (Nagamine et al. 2002). The method is based on a unique primer design that creates a “dumbbell” shaped, looped DNA structure that is self-priming. The DNA polymerase used in LAMP can displace bound strands of DNA as it advances, and thus there is no need to thermocycle the reaction for the DNA to be amplified. The cycling steps characteristic of qPCR are functionally replaced by the enzymatic strand-displacement activity of the Bst polymerase. This means that LAMP reactions operate at a single temperature (usually a single temperature 63-67^{o}C), which greatly simplifies the methodology and required instrumentation. When combined with relatively straightforward optical detection, these properties have enabled LAMP to be used for point-of-care and field diagnostics (e.g. (Calvert et al. 2017), (Cook et al. 2015), (TINY paper), (da Silva Gonçalves et al. 2019)).

The detailed mechanism underpinning DNA amplification in LAMP is complex. This arises largely from the multiple sites of polymerase initiation, itself being a consequence of using six primers to target multiple locations on the target DNA (rather than two as in PCR, **Figure 2**). The more complex elongation cycle is described in **Supplementary S1** while the Reader may find this animation more intuitive.

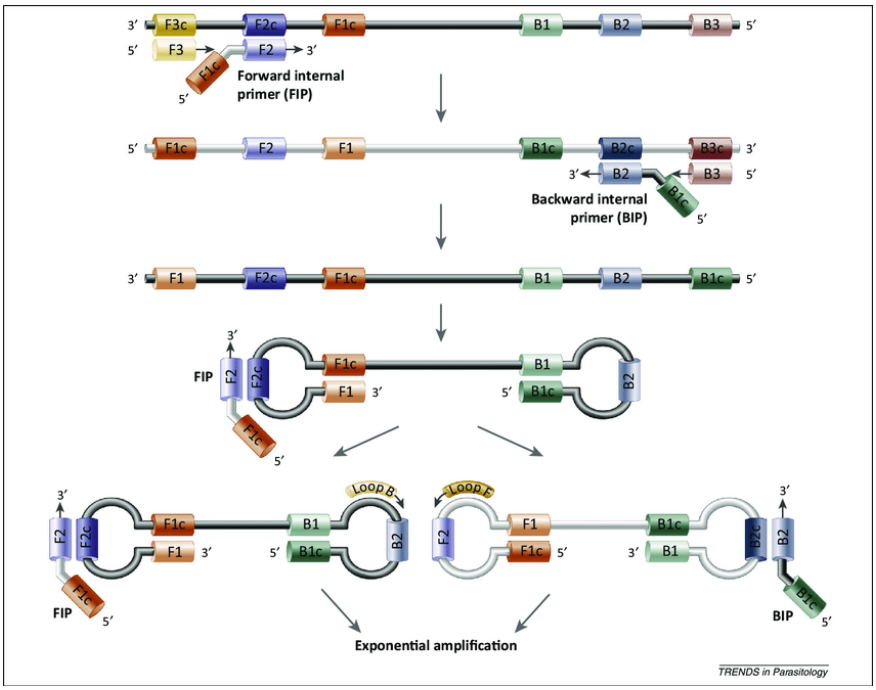


Figure 2. LAMP Mechanism. *LAMP employs two sets of primers, forward/backward internal primers: FIP and BIP, and outer primers F3 and B3, to target six distinct regions (F1c, F2c, F3c sites on one end and B1, B2, B3 sites on the other). The reaction is initiated by the binding of FIP to the F2c region on the double-stranded DNA. As the polymerase elongates the DNA from the FIP, the outer primer F3 which is shorter in length and lower in concentration than the FIP then binds onto its complementary region on the DNA and starts to displace the newly synthesized DNA. The replaced strand then forms a loop structure at one end due to the complementarity of F1 and F1c. With similar performance of BIP and B3, this results in a single-stranded double stem-loop DNA structure (so-called "dumb-bell" structure). This dumb-bell structured DNA enters the amplification cycle as it is already self-primed. Elongation by the polymerase can occur from the free 3' end of the ssDNA and from binding of the FIP/BIP primers to the single stranded loop or from the optional accelerating loop primers -- see* ***Supplementary Figure S1*** *(with permission from Alhassan et al. 2015)*

The first LAMP assays for SARS-CoV-2 were described in preprints starting in February, March, and April 2020 (Yu et al. 2020); Lamb et al. 2020 Zhang et al. 2020a ; Broughton et al. 2020; Butler et al, 2020). As demand for testing increased, laboratories normally devoted to genomics, developmental biology or plant pathogens shifted focus onto the development of diagnostic tests for SARS-CoV-2.

02.04 Specialist topic boxes: “Lenses on LAMP”

LAMP is, by its very design, adaptable. In these Topic Boxes authors shared various first-hand experiences of development and deployment and of methodological improvements

02.04.01 Topic A: Developing LAMP tests in and for resource-limited settings

LAMP testing is inherently simpler and more portable than conventional RT-qPCR and so has been of keen interest for those engaged in distributed (e.g. primary care) and low resource (e.g. developing nations) settings. We (re)introduce the (RE)ASSURED framework as criteria, such as Affordability and Robustness that diagnostics designed for resource-limited settings should aspire to. This includes the very real compromises in analytical sensitivity & specificity that are often necessary to maximize affordability and accessibility of the test to the largest patient population. These pragmatic choices are exemplified with a case study on testing in resource-limited communities in South-East Asia. Open-source methods, resources and approaches for affordable production of LAMP enzymes and hardware (Section 06.07) seek to further extend LAMP's already more affordable footprint across the globe.

02.04.02 Topic B: Hardware, reagent, and software considerations for LAMP testing

One of the most attractive features of LAMP is its inherent simplicity with reactions able to be performed with either a qPCR reader, water heater, and visual detection of color changes. And yet, there is a growing ‘middle ground’ for smaller, more portable equipment that provides the majority of the functionality at a fraction of the cost and footprint. This section highlights three such innovations. The first is a LAMP reader software application that improves the objectivity of colorimetric methods by replacing visual inspection with a dedicated App on a simple smartphone camera. Two Contributors describe the adaptation of small, semi-portable benchtop incubators and readers to LAMP testing with COVID; one (Axxin fluorimeter) coming from a medical perspective and another (BioRanger) repurposed from an original agricultural use-case. Finally, we summarize the current status of engineered mutant Bst polymerase enzymes with improved catalytic properties, underscoring the critical part played by enzymatic strand-displacement in isothermal LAMP reactions.

02.04.03 Topic C: Emerging protocols and methods from the gLAMP Consortium

The focus of this Review is measurement of SARS-CoV2 is oral samples (saliva, NP swabs etc). However, here we describe the application of direct LAMP methods to monitor SARS-CoV-2 shedding via environmental sources, employing municipal wastewater and raw sewage samples as an epidemiological tool. Monitoring and adapting primer design to emerging mutations in the SARS-CoV-2 genome detected by sequencing and surveillance studies is similarly highlighted; a topic of considerable interest at the time of writing due to the spread of the ‘UK COVID’ variant. (Pangolin lineage B.1.1.7, Nextstrain clade 20B/501Y.V1). The importance of the pre-analytical sample processing, RNA extraction and/or purification cannot be overstated.

Throughout 2020, participants exchanged (mixed) experiences of different pre-analytical methods. Two case studies provide examples of two very different types of contribution. The first is the building of a laboratory developing a saliva-based LAMP assay from "the ground up" using many of the guidelines outlined in the Review. Successfully transitioning a laboratory-developed test from bench to bedside (or school-side) is the ultimate objective of any diagnostic development process. We conclude with an account of repeat RT-LAMP surveillance testing of a single cohort of individuals (a K-12 school) with at-home participant saliva collection and onsite sample processing.

02.04.04 Topic D: Review Highlights and Takeaways

This Box highlights some important considerations for building LAMP tests, considering scale of testing, the intended use, location/setting and the budget and level of infrastructure available. It considers a series of questions related to the use-case including: What is the right test for the given application? How will the test result be used? How does the hidden cost of *not* testing affect utility? What location will the test be used in? How will frequency and time-to-results influence the design choice? What sensitivity and specificity are required -- being positive and being contagious are not always synchronous physiological states; How does the test fit into the clinical practice and triage? and How will the training and skills-level of the testing personnel influence the design? It concludes with a summary of some technical features of a "good" LAMP test methodology upon which the developer can adapt their individual approach.

Pre-analytical Sample Processing

Preanalytically, SARS-CoV-2 testing begins with a choice of sampling site/method and then a choice of viral inactivation method. Thereafter, one can choose to employ or omit an RNA 'extraction' step ("a direct method"). Generally, the former have better sensitivity and precision while the latter are less expensive, faster and logistically simpler. The unprecedented demand caused by the SARS-CoV-2 pandemic has prompted methodological refinements to allow the same (or similar) test performance to be achieved faster, cheaper and simpler.

Below, we discuss each of these component methods: sampling, viral inactivation, RNA extraction, and direct processing, and concluding with a detailed discussion of the specific issues that arise when using pH-based colorimetric LAMP assay (Figure 3). Here, we consider *viral inactivation* to be the process of preventing the replication of the virus (a safety procedure) whereas *RNA extraction* involves purposeful release of the viral RNA from the nucleocapsid and, in some protocols, its purification (an analytical procedure). Some methods that involve heating (e.g. Jon-Aritzi Sans *et al*. 2020) can fully or partially serve both purposes.

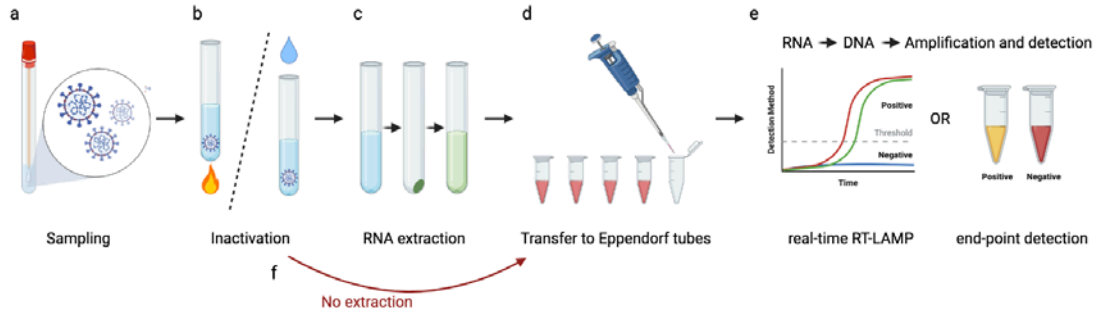


Figure 3. *Biospecimens taken from the patient (**A**) are Inactivated and with the virus being lysed through heating or enzymatic treatment, with or without the addition of chemical agents (**B**) RNA can be extracted and purified from contaminating proteins and inhibitory contaminants (**C**) or the step can be omitted ("Direct" methods, (**F**). Following transfer of the processed RNA sample into the RT-LAMP reaction mixture (**D**), detection of positive reactions can be achieved using a variety of methods (Section 5) often using real-time fluorescent or visual end-point readouts (**E**).*

03.01 Biosample Types

03.01.01 Swabs

Since SARS-CoV-2 replicates in the epithelial cells of the respiratory tract (Sungnak et al. 2020), most commonly samples for testing are taken from the upper (nasopharyngeal (NP), oropharyngeal (OP), and nasal swabs or saliva) or the lower respiratory tract (sputum, tracheal aspirate, and bronchoalveolar lavage (BAL)) (Mawaddah 2020). SARS-CoV-2 viral load in the upper respiratory tract as measured indirectly by viral RNA is elevated during the first week after the onset of symptoms, peaking at 10^4-10^7 copies/mL after 4 to 6 days (To et al. 2020). The

lower respiratory tract is more commonly sampled in symptomatic or severe cases of suspected infection although the load is highly variable. Swabs have also been used in hospital environments and subways for detecting the presence of the virus (Brune Z, *et al*., 2021), which also work for other pathogens (Rei CK, *et al*., 2020).

03.01.02 Washes

Sampling using nasal and throat washes or gargling with a simple 0.9% w/v saline or salt solution (HBSS) are also employed given their significantly less invasive and negative impact on the patient (Kellner et al. 2020; see also www.maxperutzlabs.ac.at). Encouragingly, respiratory secretions and cells removed from the upper respiratory tract contain comparable or higher viral loads than those obtained from NP or OP swabs (Mawaddah 2020). As with saliva (below), the increased viscosity of throat wash samples can be problematic during sample processing (i.e. samples cannot be pipetted and/or mucous threads pose a cross contamination risk). This can be easily addressed by incubation of the sample with fresh 5-10mM DTT for 10-15 min prior to further processing to reduce disulfide bonds.

03.01.03 Saliva

Collection of saliva can be done by the patient without the need for healthcare professional assistance, which reduces the stress on healthcare systems and alleviates the need for nasal swabs. The reference nasopharyngeal samples used at the start of the pandemic were progressively augmented with saliva specimens based on patient saliva-collection-and-drooling (not spitting), including at home collection. Using saliva specimens reduces the risk of exposure to viral droplets for medical workers, reduces the time and cost of the testing procedure compared to those for nasopharyngeal swabs.

First applied to RT-qPCR-based detection (Vogels et al. 2020, SalivaDirect), saliva-based LAMP methods have increasingly been developed although the reported diagnostic sensitivity varies between ~70-100% [Nagura-Ikeda et al. 2020 ; To et al. 2020). Comparisons between nasopharyngeal swab specimens and saliva concluded that both types of specimen have equivalent sensitivity to detect SARS-CoV-2 (Kellner, et al. 2020 ; Wyllie et al. 2020 ; Zhu et al. 2020). While collection is more straightforward than NPS, saliva is a more biologically complex and challenging sample matrix to use, particularly if ‘minimal’ nucleic acid extraction (‘Direct’) methods are used. Also, the composition of saliva varies between individuals, with researchers reporting non-specific effects of acidic saliva on the assays, an effect that must be mitigated by pre-neutralisation of the sample before LAMP testing when using poorly-buffered solutions for a pH-based readout (Nagura-Ikeda et al. 2020). The interplay of competing technical factors in the pre-analytical and analytical phases of the test is not uncommon.

Clinically, viral RNA is far less commonly isolated from urine (e.g. Kim et al. 2020; Peng et al. 2020)) and although virus has been reported in stool samples, their levels (~10^3-10^6 copies/mL)

are 10-100-fold lower than seen in oral/respiratory samples (Wolfel et al. 2020). As elsewhere, detection of the viral RNA does not necessarily correlate with clinical symptoms (Peng et al. 2020; Gupta et al. 2020). More commonly, virus is detected in excreta in the context of wastewater and sewage surveillance (Wu et al. 2020 ; see also Section 10.05.02).

03.02 Virus Inactivation methods

Infectious SARS-CoV-2 can only be worked with in high-containment BSL3 laboratories, whereas inactivation allows handling at a lower biocontainment level environment. There are three principle methods for inactivating SARS-CoV-2: (1) biological, including antibodies (Martí et al. 2020), (2) physical, including heat (Abraham et al. 2020; Cimolai, 2020; Hu et al. 2020; Jureka et al. 2020; Kampf et al. 2020; Pastorino et al. 2020; Yap et al. 2020), cold plasma (Filipić et al. 2020), and ultraviolet light (Loveday et al. (2020), Martí et al. 2020, Buonanno et al. 2020), and (3) chemical including detergents, cross-linking agents, oxidising reagents, chaotropes, alcohols and other organic solvents (Martí et al. 2020, Jureka et al. 2020, Welch et al. 2020). Frequently full inactivation is achieved by combining methods such as heat and a chaotrope (Westhaus et al. 2020).

The effectiveness of inactivation depends on numerous factors including viral and reagent concentration, protein content, and treatment time. SARS-CoV-2 inactivation is measured by the reduction of the number of infected tissue culture cells, frequently by plaque assay, or the non-detection of viral RNA when cell passaging tests are carried out (e.g. Welch et al. 2020; Kim et al. 2020). Inactivating SARS-CoV-2 is relatively easy compared with non-enveloped viruses where detergents are frequently employed to disperse or puncture the lipid membrane. However, the virus under physiological conditions has been demonstrated to maintain infectivity for weeks at room-temperature and months at 4^{o}C (Westhaus et al. 2020).

While inactivation can be achieved using a wide variety of methods, maintaining RNA integrity for subsequent detection is more problematic. RNA is among the most fragile biomolecules with rates of degradation up to a million-fold greater than DNA, which can impact downstream profiling (Li S *et al.*, 2014). Many inactivation methods including heat, chaotropes, and cross-linking reagents (such as formaldehyde) lead to loss of RNA integrity, particularly if there is an extended period between collection and molecular analysis. Commercially available reagents to inactivate SARS-CoV-2 include: 70% ethanol, 70% isopropanol, 70% acetone, Virkon (Lanxess, UK), NP-40, Triton X-100, 4M Guanidine isothiocyanate with 2% Triton X-100, Primestore MTM (Longhorn Vaccines and Diagnostics, USA), Buffer AVL and RLT with β-mercaptoethanol (Qiagen, Germany), virusPHIX (RNAssist, UK), VPSS (E&O Labs), MagNA Pure Lysis Buffer (Roche, France) and Omnigene Oral DNA (DNAgenotek, Canada) as set-out in Welch et al. 2020; see also Public Health England).

Many SARS-CoV-2 RNA purification procedures employ an initial lysis step using guanidine or TriZol which reduce or eliminate viral infectivity without affecting RNA yields or integrity (Batéjat et al. 2020). However, this step increases costs, time, uses commercial products in short supply that may be in very limited supply and several of the constituents (e.g. GnHCl or GnITC) are often incompatible with either the reverse transcription step or the LAMP reaction.

As such, physical methods that avoid use of harsh chemicals have become increasingly popular. While ultraviolet light efficiently inactivates the virus, it also reduces SARS-CoV-2 RNA detection (of note, one of the reference control reagents commonly used is gamma-irradiated virus NR-52287 from BEI Resources). Alternatively, heat can be used for inactivation, although the relatively high temperatures required (ranging from 56-95°C (Batéjat et al. 2020) may also be associated with decreased RNA integrity, particularly if a divalent metal ion chelator is not used to remove Mg^{2+} and Mn^{2+} ions. Extended inactivation (65°C, 30 min) provides > 10^5-fold reduction in viral titres (Pastorino et al. 2020) and this is the method used to prepare heat-inactivated virus by BEI Resources (NR-52286). A shorter heating step (56°C, 5 min) is not likely sufficient for complete SARS-CoV-2 inactivation (Westhaus et al. 2020).

In summary, while SARS CoV2 inactivation is relatively straight-forward, the challenges rest on the delicate balance between clinically complete viral inactivation, maintenance of RNA integrity, use of simple methods, and the compatibility of the viral inactivation method with the down-stream analytical RT and LAMP steps.

03.03 RNA extraction

03.03.01 The rationale for RNA Extraction

Depending on the application and use-case, there can be compelling reasons to include an RNA extraction step, as it holds certain clear advantages over minimally processed ('Direct') samples.

First, patient samples have the potential to be highly heterogeneous – the degree being dependent on the sample type. NP swabs, and arguably other types of swabs, are less variable between individuals – likely because they are diluted in a common medium such as Viral Transport Media (VTM), saline, or Phosphate-Buffered Saline PBS.[1] Saliva, on the other hand, is heavily influenced by factors such as diet, time of day, smoking, and behavior. Nucleic acid purification, which aims to remove RNA from the patient specimen and reconstitute it in a common matrix, thus eliminates confounding factors that may be present in minimally processed specimens. This is particularly important in colorimetric LAMP, which relies on either pH or free magnesium content as a read-out, and is therefore potentially subject to greater interference from confounding substances.

Second, nucleic acid purification serves to concentrate RNA to improve diagnostic sensitivity by ~ 1-2 $\log_{10}$ units depending on the original specimen, the method of purification, and the

downstream assay. Given these two advantages and despite the promise of (and enthusiasm for) Direct methods, for some use-cases it may very well be worthwhile to introduce a nucleic acid purification step. Indeed, out of the ten RT-LAMP-based methods granted Emergency Use Authorization (EUAs) as of April 2021, five use some form of nucleic acid purification (Table 1). In the following section, we describe several open-source low-cost methods for nucleic acid purification used by several of the authors as simple yet effective alternatives to commercial RNA extraction kits.

03.03.02 Silica-based methods

The propensity of nucleic acids to bind to silica in alkaline and high salt conditions was first identified when DNA was demonstrated to bind to glass fibers when solubilized in sodium iodide (NaI). Since then, silica matrices have seen widespread adoption in molecular labs – most commonly in the form of silica spin columns. Although effective, the additional cost and centrifugation requirement of spin columns are antithetical towards the development of an accessible low-cost test. Nevertheless, many efforts have sought to exploit this property of silica in a more affordable fashion.

Researchers returned to the use of a silica particle suspension (glass milk) in conjunction with NaI for purifying RNA from either NP swabs in saline/PBS or saliva (Rabe and Cepko, 2020). They found that their glass milk-NaI purification achieved sensitivity down to 1 copy/µl in 500µl of material in a pH-based colorimetric RT-LAMP assay at a processing cost ~ $0.07 per sample. Although the original method demands several centrifugation steps, they found that centrifugation can be substituted by a 5-10 min settling step when using NP swabs in saline/PBS. However, saliva proved too viscous to be amenable towards this settling step.

However, the centrifugation demands of their protocol are relatively light, and thus can be theoretically fulfilled through low-cost alternatives such as the Paperfuge (Saad Bhamla et al., 2017). Li et al., 2020 validate the glass milk protocol with saliva on their open source Handyfuge device, which they estimate at under $5 to construct. Intriguingly, Garneret et al. 2021 repurpose the concept of the silica gel membrane found in spin columns for use in a folding card device. They embed a silica membrane on one side of the folding card and freeze-dried fluorescence-based RT-LAMP reactions on the other side. NP swab samples are injected into the silica membrane before being washed and eluted into the freeze-dried RT-LAMP reactions. Although their manuscript does not describe the chemical parameters of their assay, the principle of the silica membrane likely operates in a similar fashion to Rabe and Cepko's glass milk purification (Rabe and Cepko, 2020).

03.03.03 Magnetic bead-based methods

Although glass milk likely represents the most inexpensive option for nucleic acid purification, it does not reach scaling as well as other platforms. If used with centrifugation, glass milk

purification becomes largely incompatible with most forms of liquid-handling automation; whereas if it is used without centrifugation, sample processing time increases by upwards of 30 minutes. Magnetic beads operate on the same principle as glass milk – in fact, they usually consist of ferrite cores with a silica or carboxyl coating -- but require only a simple magnet for bead separation. Although more expensive when purchased from commercial suppliers (see below), magnetic beads offer facile, centrifugation-free handling and improved automation compatibility.

Multiple groups have found commercial products that are compatible with RT-LAMP. Klein et al. (2020) present a protocol using SiMAG-N-DNA magnetic beads combined with a homemade Guanidinium isothiocyanate (GITC) solution to purify nucleic acids from NP swabs for use in both pH-based colorimetric and fluorimetric RT-LAMP assays. Kellner et al. 2020 demonstrated the capacity of AmpureXP RNAClean to purify RNA from multiple sample types for use in magnesium-sensing colorimetric RT-LAMP. Altogether, they validated NP swabs, gargle, and sputum samples mixed with Sputolysin (buffered DTT) solution and estimated their limit of detection from all sample specimens at 10 copies/µl.

An open-source alternative to AmpureXP beads for purification from saliva has been described (Yu et al. 2020), reducing processing costs from ~$1/sample per sample to ~$0.20, processing time (20 mins to 10 mins) and improving yield from saliva relative to the commercial option, achieving a limit of detection of 3.7 copies/µl in pH-based colorimetric RT-LAMP. Interestingly, they found that typical magnetic bead-based purification, which involves removing supernatant from the beads during washing, introduced too much carryover of flocculent matter from saliva, which interfered with the subsequent RT-LAMP reaction. Instead, they used a 3D-printed magnetic stick with a disposable tip to remove the beads from the supernatant, which selectively removes the beads over the flocculent matter. Bektaş et al. (2021) also developed a magnetic stick. However, unlike Yu et al. who found it to be necessary for purification from Saliva other researchers (Bektas et al. 2021) were motivated to use a magnetic stick to produce a test that eliminated micropipetting and so was compatible with home testing.

In addition to solid-phase extraction through silica or carboxyl coatings, magnetic beads have also been used to purify nucleic acids through hybridization. Bokelmann et al. 2021 binds biotinylated oligonucleotides complementary to the Orf1b and N genes to streptavidin coated magnetic beads and use them to purify specific regions of the SARS-CoV-2 genome from gargle samples in sterile water. They estimate their limit of detection to be 5-25 copies/µl. Whether this method proves to be substantially advantageous remains to be seen, but unlike solid-phase extraction methods, it does not require high salt conditions to bind nucleic acids, thus obviating the need to wash off said salt prior to input into RT-LAMP.

03.04 Direct Methods

The preceding section makes the case for nucleic acid purification in diagnostic testing to normalize potentially heterogeneous patient samples while also increasing sensitivity through nucleic acid concentration. However, despite widespread use, the necessity of such a cost- and time-intensive procedure may be an unchallenged axiom rather than an empirically determined need. Given the unprecedented scale of testing demanded by the SARS-CoV-2 pandemic, many have sought to determine whether nucleic acid purification may be eliminated while maintaining (sufficient) sensitivity and specificity across different patients ("Direct methods").

Any Direct method must nevertheless still fulfill all four pre-analytical criteria.

1. Samples must be rendered non-infectious, to ensure the safety of the technician or clinical staff;
2. Viral RNA must be released from the viral envelope and made available for assaying;
3. RNAses present in the patient sample must be inactivated, so that they do not reduce the amount of available RNA prior for assaying;
4. The sample must be compatible with the assay in question by minimizing the interfering impact of compounds endogenous to the sample.

Samples processed under these criteria are referred to as 'minimally processed samples' and we provide an overview of such methods below.

03.04.01 Heat and chemical agents

Perhaps the most intuitive means to fulfill the first two criteria is to denature undesirable proteins with heat. Indeed, treatments from 60-95˚C for various amounts of time have been demonstrated to be effective at eliminating SARS-CoV-2 viral replication (see 03.02, Inactivation above) and releasing RNA. At least three studies have found that direct heat treatment of both saliva and NP swab samples is compatible with downstream RT-LAMP, representing the simplest and most minimal form of pretreatment.

However, RNAses are known to resist denaturation at even boiling temperatures and are capable of refolding following denaturation. Therefore, heat treatment of patient samples is often augmented with reducing agents, either dithiothreitol (DTT) or preferably the more stable tris(2-carboxyethyl)phosphine (TCEP). Both RNA preservation agents exert an inhibitory effect on RNAses through cleavage of disulfide bonds, are compatible with downstream enzymatic assays, and both have their activity enhanced at the high temperatures used for heat inactivation. Many studies have used TCEP to great effect, finding it to be an effective reagent in preprocessing saliva and swab specimens alike (Cepko et al. 2020).

03.04.02 Proteinase K and Surfactants

An alternative to heat denaturation of proteins is proteolytic digestion. It is theoretically effective against even the most heat stable of proteins and it precludes the possibility of refolding and like TCEP, it stands to offer protection against RNAses. Many approaches have validated the use of Proteinase K as an effective reagent for processing saliva and swab specimens. In general, this preprocessing step involves digestion for 5-15 min at 37-65˚C, where Proteinase K remains active, followed by protease inactivation at 95˚C for 5-10 minutes to ensure it does not interfere with downstream reactions.

In the event that the second 95˚C incubation step is undesirable, thermolabile Proteinase K, which is active at 37˚C and is inactivated at the 65˚C used for LAMP assays, can be used. Its use decreases the potential for tubes to 'pop' and create cross-contamination during elevated heating and simplifies the protocol although there is comparatively less experimental validation with the thermolabile variant (Wei et al. 2020), possibly due to its higher cost. Further validation and increased accessibility of thermolabile Proteinase K may be useful for future diagnostic efforts. In addition one study has validated the effectiveness of semi-alkaline proteinase (SAP) on saliva samples as a possible alternative to proteinase K.

Mild surfactants can be used to disrupt cell membranes and release intracellular materials. Non-ionic detergents, with uncharged hydrophilic head groups such as Triton X-100 and Tween-20 are routinely used to lyse cells (Johnson, 2013) and are components of some RT-qPCR (e.g Ranoa et al. 2020) and RT-LAMP (Wei et al. 2020; Azmi et al. 2020) methods. However, some have observed that the addition of 0.1% of Triton X-100 (but not the more gentle surfactant, Tween-20) during lysis of negative SARS-CoV-2 saliva samples resulted in RT-LAMP false positive read-outs (Bendesky, unpublished results) while others have noted the need for removal (i.e. washing) of Triton-X100 in some applications (Ma et al. 2020 c.f Azmi et al. 2020). Finally, Bendesky and colleagues (unpublished) report that the subsequent RT-LAMP reaction is more sensitive if Tween-20 is combined with 'regular' (but not thermolabile) proteinase K during the extraction; presumably the protease degrades viral proteins that have become accessible after lysis of the lipid envelope.

03.04.03 Chelating agents

Rather than degrading RNAses, RNA may also be protected by denying RNAses the cofactors required for their function, such as magnesium. TCEP, in conjunction with the metal ion chelator EDTA, exhibits a partial protective effect against some but not all RNases. Since Mg^{2+} is also a required cofactor for RT-LAMP, this limits the allowable carryover of EDTA into the RT-LAMP reaction. Therefore, other researchers (Flynn et al. 2020) added a chelating resin (Chelex-100) to saliva prior to a 95˚C heat treatment, pelleted the resin via centrifugation and used the supernatant as their RT-LAMP input. While this benefits from the chelating effect of Chelex-100 without carrying over into the downstream reaction, it adds the requirement for a simple centrifugation step which may be undesirable in certain use-cases. Recently, Howson et al. 2021

published encouraging results with a centrifugation-free saliva processing method involving Sputolysin (buffered DTT), heating and Chelex-100 resin which resulted in a notably low false positive rate (<1:3000) using a fluorescent intercalation detection scheme.

03.05 Considerations for pH-based colorimetric RT-LAMP

Of the two main colorimetric methods used to monitor LAMP reactions (changes in pH and $[Mg^{2+}]_{free}$), the pH-based readout is more commonly found in the literature. Here, the reaction changes color as dNTP incorporation by the polymerase releases protons, thereby acidifying a weakly buffered reaction medium containing a pH indicator (see Section 5, Amplicon Detection). As such, the initial pH of the reaction is of critical importance. When coupling a colorimetric readout with RNA purification, assuring sample compatibility is simply achieved with a suitable buffer to reconstitute the RNA. However, when working with minimally processed samples, the sample itself must often be chemically adjusted to a suitable pH. To this end, many protocols include sodium hydroxide (NaOH) – especially those that use TCEP, which is inherently acidic.

For swab samples, which are typically reconstituted in a diluent or transport media, the contribution of the media on pH can be expected to prevail over the contribution from the respiratory specimen – meaning that one can expect a relatively homogenous spectrum of pH values, making it easy to ensure consistent compatibility with pH-based RT-LAMP methods (Butler et al. 2021). However, when assaying saliva samples directly, the saliva itself becomes the sole determinant of the initial pH. Saliva occupies a wide pH range and is easily influenced by factors such as time of day and diet which confounds efforts at developing a universal solution for ensuring saliva compatibility with colorimetric RT-LAMP. Efforts to test this have suggested final concentrations of NaOH ranging from 1.2mM to 1.45mM, but optimization is still required. At high concentrations of NaOH, alkaline samples exhibit a reduced color change, while at low concentrations of NaOH, acidic samples exhibit color change prior to incubation. This may be an acceptable compromise in low-throughput environments, where expert subjective determination and/or resampling for a compatible specimen are possible. However, minimally processed saliva has limited compatibility with pH-based colorimetric RT-LAMP in a mid-to-large scale diagnostic environment.

Partly for this reason, some prefer the use of alternative colorimetric dyes that operate on a different principle and in a buffered solution (e.g HNB for Mg^{2+}) or use a fluorescence-based readout (Section 05). However, it is worth noting that although the issue of compatibility is most obvious when developing a protocol for pH-based colorimetric RT-LAMP, it is likely a factor in these other assays as well. The full chemical composition and degree of variation in respiratory samples between individuals is not well understood, nor is their interaction with nucleic acid detection technologies. Such interaction variation in clinical samples suggests that certain sample types may be less amenable than others to detection, with corresponding propensities for false

positive or false negative results. In the absence of more complete understanding of the interdependencies, as well as how this may change with variants of a virus (Alpert T, *et al*., 2021) or regional differences (Danko D, *et al*., 2021) this spectrum of compatibility tends to be functionally replaced in favor of a binary mode of interpretation: either the processing "works", or it does not. The degree to which patient sample heterogeneity affects purification-free testing is currently incompletely understood. Although it is an acceptable, and many would argue, necessary compromise given the urgency of the pandemic and the need for widespread and rapid testing, future efforts may be well served by a more systematic characterization of respiratory samples. As elsewhere, it is probable that such research will indicate that there is not a one-size-fits-all minimal processing solution.

Table 1. Summary of Literature Review of Different LAMP Processing Methods.*indicates sensitivity reported using combination RT-LAMP/RPA with CRISPR-Cas detection; else only RT-LAMP.

Processing method	Author	Approximate Sensitivity or Limit of Detection	Sample type
65C for 30 min	L'Helgouach et al.	73% Sensitivity at qPCR Ct < 35.	Saliva
95C for 15 min	Alekseenko et al.	Sensitivity comparable to qPCR at Ct = 20-25	NP Swabs
95C for 5 min	Thi et al.	Sensitivity comparable to qPCR at Ct = 25-30	NP Swabs
Chelex-100, DTT	Howson et al	Sensitivity comparable to qPCR at Ct = 25-30	Saliva
Chelex-100	Flynn et al	100 copies/µl	Saliva
Proteinase K	Ben-Assa et al.	Sensitivity comparable to qPCR at Ct = 28	NP Swabs in UTM, Saliva
Proteinase K	Azmi et al.*	10 copies per reaction	Saliva

Proteinase K (QuickExtract)	Joung et al.*	100 copies per reaction	NP Swab in VTM
Proteinase K (QuickExtract)	Nguyen et al.*	1 copy/µl	NP Swabs
Semi-alkaline proteinase	Yamazaki et al.	170-230 copies/µl	Saliva
TCEP/EDTA (Quick Extract)	Agrawal et al.*	40 copies/µl	Saliva
TCEP/EDTA or DTT/EDTA	Rabe et al.	50 copies/µl	Saliva, NP Swabs
TCEP/EDTA	Sherrill-Mix et al.	100 copies/µl	Saliva
TCEP, Proteinase K	Yang et al.	200 copies/µl	Saliva
TCEP, RNASecure, Proteinase K	Lalli et al.	25 copies/µl	Saliva
Thermolabile Proteinase K	Wei et al.	2.5 copies/µl	NP Swabs in VTM

Target Amplification

Nucleic acid amplification tests rely on three molecular steps: i. sequence-specific recognition of the target through primer base-pairing; ii. enzyme-catalyzed amplification of the targeted DNA segment(s) and iii. detection of amplified products (amplicons). For SARS-CoV2, as the target is RNA, it must be initially reverse-transcribed into complementary DNA before amplification (unless using direct RNA sequencing, as in Liu H, *et al*., 2019). The following section reviews general design of primers, reaction optimization, possible assay configurations, controls, and validation strategies. Figure **4** depicts a flow chart of the general means to design RT-LAMP primers and optimize reactions applied to SARS-CoV-2.

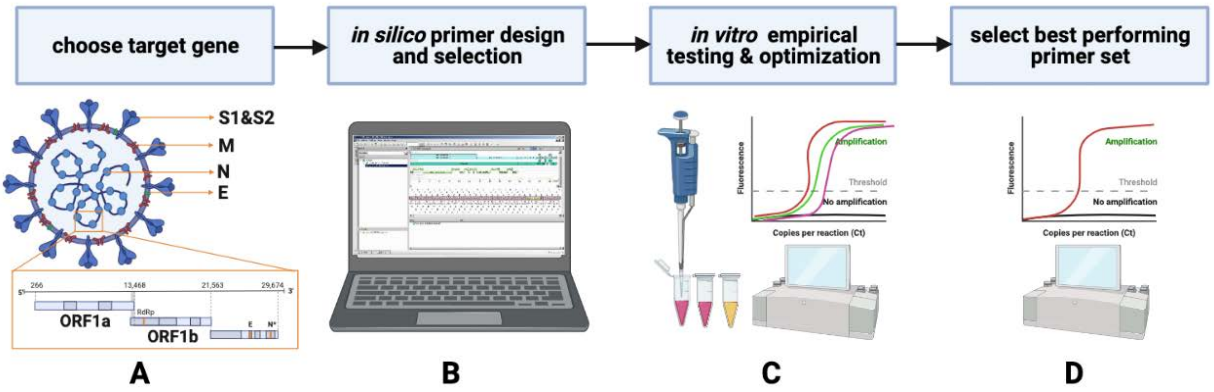


Figure 4 *A flowchart for SARS-CoV-2 RT-LAMP primer design and selection. Having chosen a preferred viral target genomic sequence based on e.g abundance, mutagenesis consideration* ***(A)*** *primer sets are designed and selected in silico****,*** *considering potential primer-dimers or other undesired interactions, inclusivity across SARS-CoV-2 variants, and exclusivity from other coronaviruses (i.e. MERS) or species* ***(B)****. Having selected promising primer sets in silico, empirical testing* ***(C)*** *(time-to-threshold, limit-of-detection, etc.) and reaction optimization in the laboratory identifies the set(s) with the desired empirical properties. (****D****).*

04.01 Primer selection

Specific and sensitive target amplification of SARS-CoV-2 by RT-LAMP requires selection of optimal primers sequences (based on the target genome sequence) and reaction conditions (often based on literature precedent from other RT-LAMP assays). The SARS-CoV-2 genome comprises ~30,000 RNA bases, encoding genes for structural (e.g nucleocapsid, N., Spike S) and non-structural (e.g. RNA-dependent RNA polymerase, RdRp) components of the virus (Figure 5). Early diagnostic efforts (for example, Corman et al. 2020) were based on the published sequence (Wu et al. 2020) and utilized several potential genes (ORF1a, ORF-1ab, RdRp, S, N, and E), which have become the focus of RT-LAMP and other nucleic acid tests (**Table 2**). Since the N gene is at the 3' end of the viral genome, it is contained in all coronaviral, sub-genomic RNAs (expressed as a nested set) and is the most abundant viral RNA in infected cells (Finkel et al. 2020). Consequently, while N gene is a good target based on abundance, areas of the viral

genome (e.g. Spike gene) harboring low frequency but functionally important mutations (see nextstrain.org, 10.xx, Topic Box C) might be predicted to be useful targets for examination of SARS-CoV-2 evolution.

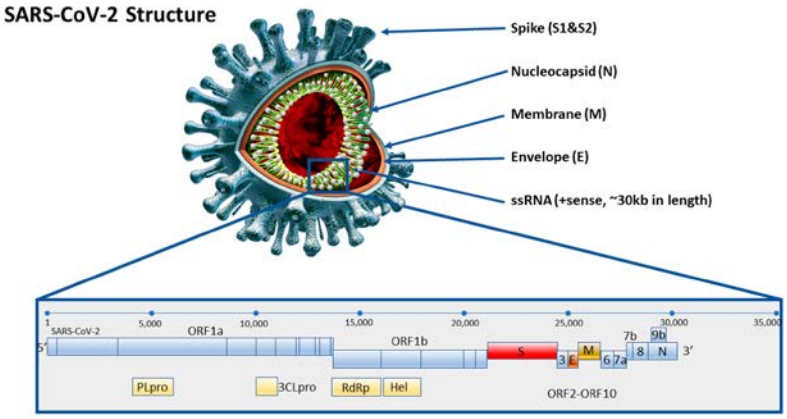


Figure 5 Representation of the physical and genomic RNA structure of SARS-CoV-2. *The genome of the virus is shown below, and a rendering of the viral structure is shown on the top.*

The original LAMP publications (Notomi et al. 2000, Mori et al. 2001) used four primers targeting six discrete sites on a sequence of interest (two of the primers, FIB and BIP, are effectively 'dual-function primers' in that they contain two discrete binding sequences, see Figure 2). The addition of two 'loop' primers (Nagamine 2002) provide additional sites for the Bst polymerase to propagate DNA amplification with the consequence that the time-to-positive for many tests is reduced to well within 30 minutes. Robust reactions with comparatively little sensitivity to matrix interference (Francois 2011, Hu 2017. Kaneko 2006 - see also Section 03.03-03.04) combined with rapid supra-linear factorial amplification kinetics allow target detection with a sensitivity comparable to RT-qPCR (ElviraGonzalez et al. 2017; Lucigen 2018). Information on previously reported sets is collated in **Table 2** and **Supplementary Table 1**).

04.01.01 *In silico* Primer Design & Selection

Several open-access software tools have been developed to help design multiple primers, each of which must bind simultaneously but uniquely to the target. They predict the potential for undesirable base-pairing interactions between primers and for the unwanted formation of secondary structures such as stem-loop structures in the primers.

The first steps are to select the target gene and ensure that unique sequences can be targeted (**Figure 4A-C**). BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to ensure primer sequences are non-repetitive and unique compared to related pathogens (e.g. MERS) or to the human genome is essential. Several programs exist to design LAMP primer sequences for a target of interest. The more commonly used programs are 1. PrimerExplorer V5 alone or with the MorphoCatcher (Shirshikov et al., 2019) web plug-in which can scan individual 2000 bp

regions or alignments of such regions, 2. a more recent alternative from NEB (Primer Design), and open source programs 3. LAVA (Torres et al. 2011) or 4. GLAPD, (able to accommodate full genomes as the input) (Jia et al. 2019). Due to the complexity of the primer design and large number of primers, the default settings in the chosen primer design software tools are generally a good starting point for many users.

One needs to ensure the target sequences are not predicted to cross-react with, and hence amplify, target nucleic acids from related coronaviruses (e.g. MERS, Shirato et al. 2014) or other species (especially human). In addition to assessing *exclusivity* (that primers only bind to the pathogen/target region of interest), it is also necessary to evaluate their *inclusivity* by comparing them against known variation in the targeted region (Corman et al. 2020, Sapoval 2020). This is particularly (and increasingly) important as new variants of the virus emerge (https://www.gisaid.org/; Nextstrain.org) to ensure newly arising viral variants are still detectable with these primer sets, including for low-input samples (Parker CW, et al., 2020). From an inclusivity perspective, LAMP is, in general, quite tolerant of small changes to the target sequence although the converse if that it is more difficult to create specificity for SNPs/variants at the level of primer selection (see Topic Box C, 10.16).

04.01.02 Empirical Testing

Using these programs, several (2-5) basic primer sets (FIP/BIP, F3/B3) are typically designed for each target and then tested experimentally initially separately (i.e single-plex) using simpler DNA/RNA controls as the template, screening for amplification speed (shortest time-to-positive), sensitivity (lowest limit of detection, LoD), and a low frequency of spurious amplification in non-template controls (NTC, e.g. water, Figure 4D-E). Initial tests ideally use 'real time' amplification conditions, for example with an intercalating dye (e.g. SYTO9) or other continuous readout (Section 05.01).

After the best of the four core LAMP primers are chosen, two loop primers designed to enhance specificity and accelerate the amplification (Nagamine et al. 2014) are typically included. Further *in silico* tests with a variety of *multiple primer* analyzers (e.g Primer Analyzer, Oligo Calc, OligoAnalyzer) allow assessment of potential base-pairing interactions between and among chosen primers, to rule out undesirable formation of stem-loops or unintended complementary base-pairing especially at their 3' ends. These additional checks seek to maximize the specificity of amplification by the primers and avoid cross-priming (see also **Supplementary Materials S2**).

Despite the undoubted necessity and value of *in silico* design, the most sensitive and specific set of primers is selected empirically. The best primer sets can detect single-digit copies of their target per reaction within about a half hour; a fit-for-purpose assay for many applications will be able to detect several hundred target copies per reaction. Enhanced sensitivity has been reported

by including forward and backward 'swarm' primers targeting areas upstream of the FIP/BIP hybridization sites (Martineau et al. 2017), an approach achieving single digit/reaction sensitivity for SARS-CoV-2 with a HNB/Mg^{2+} colorimetric readout (Lau et al. 2020).

04.01 03 Troubleshooting

If a complete primer set with acceptable properties cannot be found, or if improvements are needed after empirical testing (see below), it is possible to find alternative sets in the region of interest, the AT/GC content can be increased or other individual parameters (melting temperature, region length requirements, spacing, etc.) can be readjusted. Early studies with LAMP included 4 dT's that separated the two ~ 20-25 nt component F1c/B1c and F2/B2 regions that make up the longer FIP/BIP primers. This approach is sometimes still used (e.g. As1e primer set, Rabe and Cepko, 2020) and remains an option since the TTTT sequence is predicted to disrupt potential secondary structures. When ordering primers, HPLC purification is particularly recommended for the longer FIP/BIP primers which, while more expensive than purchasing with a simple desalting, eliminates truncated primers formed during synthesis that can reduce sensitivity and reaction efficiency. Therefore, if the first set of poorly performing primers were not HPLC purified for cost reasons, this is one avenue to explore.

While the majority of LAMP assays use a relatively standard range for the absolute and relative concentrations of each primer (0.2uM F3/B3, 0.4uM LoopF/B, 1.6uM BIP/FIP), this practice is common but not absolute (e.g. Allgower et al. 2020). For example, reducing the F3/B3 concentration relative to the other four primers can sometimes improve efficiency (S. Chittur, pers. comm). Alternatively, doubling the concentration of loop primers can increase sensitivity while in other cases, somewhat counter-intuitively, complete omission of one of the loop primers can also enhance assay specificity and/or amplification efficiency (S. Chittur, pers. comm). As with PCR, modifications to primer concentrations and altering reaction conditions ([Mg^{2+}, dNTP, buffer type, addition of 'enhancing' additives such as betaine, DMSO, and notably, guanidine hydrochloride, Zhang et al. 2020c) can lead to improved assays. However, many researchers prefer the convenience (albeit at increased cost) of using a standard pre-prepared master-mix from a commercial supplier of LAMP reagents since they have already undergone assay/buffer optimization.

If open-source enzymes are used, (see Section 06.07) this will necessarily require individual optimization for each important reaction variable. It is clear that some enzymes and/or their engineered derivatives have altered biochemical properties that can be affected by solution conditions (e.g salt concentrations, Topic Box B). As in many cases with LAMP technology, the researcher can choose to take a more 'active' bottom-up-build approach to developing a test/primer set or a more 'passive' kit-based approach based on literature precedent, again depending on their expertise, needs, timeframe and budget.

04.01.03 Multiplexed primer sets

Multiplexing is performed in one of two ways depending on the test's objective. If the objective is to enhance genomic coverage, speed and/or sensitivity of LAMP reactions, one approach is to combine primer sets for multiple genes from the target, for example Gene N, E, and Orf1a from SARS Co-V-2. In this configuration one does not seek to distinguish which particular gene/amplicon is being amplified within the *'combiplex'* (Butler et al. 2021, Zhang et al. 2020b, Dudley et al. 2020). In contrast where multiple targets are to be distinguished in a single reaction (e.g SARS-CoV2 and Influenza or SARS-CoV2 and human internal extraction control genes, Zhang et al. 2020c) then sequence-specific multi-color detection must be employed for such a bone fide *multiplexes* (see Section 05.02).

In either case, the design phase necessarily becomes more complex due to the combinatorial potential for base-pairing interactions between primers. Depending on the individual sequences used, combining two (12 primers) or three (18 primers) targets into a single reaction can be achieved, though potential for spurious amplification in non-template controls should be carefully monitored. Non-specific amplification curves are typically shallower (reflecting less efficient amplification), can often be multiphasic and yield a melt curve that has a maximum slope (-dF/dT) that is sometimes noticeably (~2-3^{o}C) shifted away from that of *bone fide* amplicons. Of course, the only true means to distinguish template amplification from non-specific amplification is ultimately to perform sequencing (Section 05.03) though that is rarely used in routine practice for most/many labs.

04.02 Enzymes

04.02.01 Strand-displacing DNA polymerase

The DNA polymerase used for DNA amplification in LAMP reactions must have both DNA template-dependent 5'→3' polymerase activity but also strand displacement activity at a single elevated temperature (60-74^{o}C depending on enzyme, usually 63-65^{o}C). The most commonly used polymerase is Bst DNA polymerase from the thermophilic bacterium *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*, hence Bst) (Kiefer 1997, Phang 1995). More specifically, the Bst-LF (large fragment) lacking the smaller N-terminal domain responsible for 5'-to-3' exonuclease activity is utilized due to its ability to carry out strand displacement synthesis in the absence of the nuclease activity (Maranhao 2020).

Engineered enzymes capable of nucleic acid amplification have recently been reviewed (Yasukawa K, et al., 2020). Thermophilic versions of DNA polymerases, capable of resisting high temperatures (e.g. during sample treatment), have been evolved *in vitro* via emulsion-based directed evolution ("high-temperature isothermal compartmentalized self-replication", Milligan 2018) with some being used successfully in RT-LAMP (Alekseenko et al. 2020). Further

improvements to increase purification yields include replacing the N-terminal domain of Bst with the small F-actin binding protein villin, a modification which improved folding and protein solubility (Maranhao 2020). Taking a different approach, Ignatov et al. (2014) developed a derivative of the classic Taq polymerase (SD DNA polymerase) possessing stronger strand displacement activity and thus suitable for LAMP. Lucigen's (https://www.lucigen.com/) LavaLAMP™ uses the OmniAmp polymerase (developed from PyroPhage3173 DNA polymerase, Chander et al. 2014), which operates at higher temperatures (68-74°C), potentially leading to improved sensitivity, specificity and in some conditions, faster reaction time (Lucigen LAMP Information).

Other commercial developments include *in silico* design and evolution to improve amplification speed, salt tolerance, thermostability and yield of LAMP reactions. For instance, New England Biolabs (https://www.neb.com) created two Bst variants: (i) Warmstart Bst 2.0® allows preparation/assembly of reactions at room temperature since the polymerase is prepared with a reversibly-bound aptamer, which inhibits polymerase activity at temperatures below 45°C (NEB manual, 2017) and (ii) Bst3.0, designed to have increased RT activity to enable one-enzyme amplification of RNA targets without addition of reverse transcriptase (see below).

04.02.02 Reverse Transcriptases and Dual-Function Polymerases

For SARS CoV-2 detection, the reaction needs to detect the viral positive-sense RNA and thus reverse transcription of RNA to cDNA is required prior to amplification. By simply altering buffer conditions, some polymerase can act as both a reverse transcriptase (RT, RNA-dependent DNA polymerase) and DNA polymerase (Bhadra 2020b, Bhadra 2020c, HAWKZ05 (Roche Diagnostics)). More purposefully, dual function DNA polymerases with RT activity have been developed as protein chimeras (Schönbrunner2006) while a Bst variant with enhanced RT activity has been developed (NEB, Bst 3.0).

There is general consensus that diagnostic assays using a single, dual-function enzyme system are less sensitive than those employing dedicated RT and strand-displacing DNA polymerases in combination. If the two enzymes are to be combined and if both reverse transcription and DNA amplification steps are to be performed at a single temperature, the RT must retain sufficient activity at 60-65ºC. Historically, Avian Myeloblastosis Virus (AMV) RT was the enzyme of choice for RT-LAMP and this remains a viable option. More recently, RT from Human Immunodeficiency virus (HIV) has been demonstrated to be a functional open-source alternative (Kellner 2020) to AMV/MMLV, and even used to detect RNA modifications (Saletore *et al*., 2012, Novoa *et al*., 2019), but other options include engineered RTs such as WarmStart RTx from NEB (Zhang/Tanner 2020a/b) and SuperScript IV RT (Park 2020).

04.02.03 Other considerations

i. Storage: Lyophilised mixes for LAMP reactions were first described in studies to detect the Newcastle disease virus (Pham et al. 2005). The approach, pioneered commercially by Eiken with freeze-dried pellets being placed in the caps of PCR tubes, has been followed by Optigene (http://www.optigene.co.uk/; http://www.optigene.co.uk/polymerase-selection-guide/).

ii. Two-temperature RT-LAMP: If RT-LAMP reactions are intended to be performed in a laboratory setting with a conventional qPCR thermocycler, a 'one-pot -two-temperature' design can sometimes lead to increased sensitivity whereby the RT reaction is allowed to proceed at a lower temperature (i.e. 10-20 min at 55^{o}C) than polymerisation (~65^{o}C) (Ganguli et al. 2020; Bektas et al. 2021).

iii. Open Source Enzymes: Finally, to both reduce costs and improve the supply chain in the face of high demand, researchers (notably in developing countries) have increasingly begun to explore in-house production of enzymes required for RT-LAMP. These 'DIY alternatives to commercial enzymes include reverse transcriptases and DNA polymerases,(Alekseenko et al. 2020, Kellner et al. 2020, Sherril-Mix et al. 2020; Bhadra 2020 Bst-LF lysates).See also **Topic Box B** (Bst enzyme engineering) and Section 06.07.03 (Open Source Bioware).

04.03 Controls & reference standards

As a matter of routine, positive and negative control reactions must always be run in parallel (SEQC/MAQC-III Consortium, 2014, Foox J, et al., 2021) with each unknown sample (or each test-batch of unknowns depending on the scale of testing). If amplicons are not detected in the positive control, the functionality of the reaction mix may be suspect; if amplicons are detected in the negative control, spurious (mis-priming) amplification or cross-contamination (with amplicon or spurious DNA) could have occurred.

04.03.01 Negative controls and contamination mitigation

Non-template controls (NTC) most commonly use DNA/RNA-free, nuclease-free distilled, deionized water or Tris-EDTA (TE). While auto-amplification in NTC wells from primer:primer interactions can be observed after prolonged incubation ($\gtrsim$ 40-60 min), if amplicons are detected in NTC wells significantly earlier in the reaction time course ($\lesssim$ 40 min), cross-contamination with previously synthesized amplicon product could also be suspected. Given the exceptionally large amount of multimeric amplicon formed in LAMP (*c.f* PCR) reactions and their particular stability, the potential for cross contamination of amplicon from one run to the next is very significant. Laboratories should employ strict inventory/storage procedures (e.g. single use aliquots) and location- or isolation-related standard operating procedures. Thus, isolation of 'clean' areas (for aliquoting primers and assembling 'mastermix' solutions) from sites where amplicons could be present is strongly recommended; researchers often employ separate laboratory coats for each area. Whatever the procedures applied, maintaining a heightened awareness of the potential for contamination is essential in all LAMP laboratories.

Even great care to avoid cross-contamination may still be insufficient. The dUTP/UDG (or UNG) system (Kim et al. 2016) incorporates dUTP into LAMP amplicons in 'Experiment N'. If such labelled-amplicons are unintentionally carried over into 'Experiment N+1' then they are cleaved by the UDG enzyme present in the LAMP reaction mixture before being spuriously amplified (Kellner et al. 2020, Figure 6). The dUTP/UDG system can greatly reduce such risk, with limited impact on the RT-LAMP reactions (a marginal loss in sensitivity), but they cannot be completely removed. Confirmation that the amplicons are indeed specific for the target of interest can be accomplished by direct sequencing, but melting curve analyses can also characterize different amplicon products in a multiplex reaction (e.g. Sherril-Mix, 2020), without resorting to the opening of post-amplification tubes.

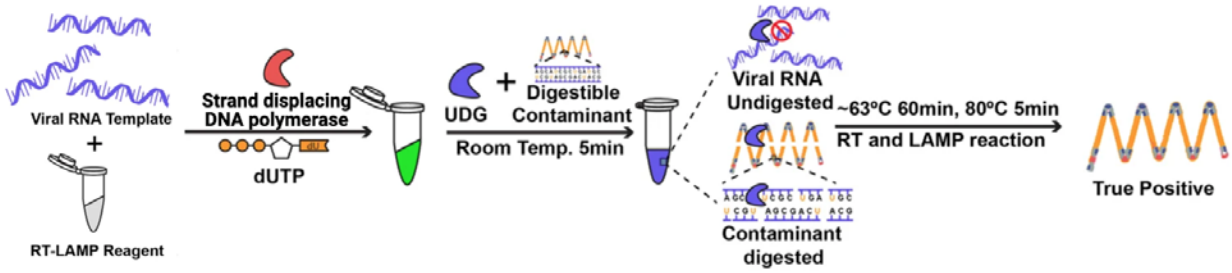


Figure 6. Uracil-DNA-glycosylase (UDG) supplemented RT-LAMP: *The system, (Kim et al. 2016) removes carryover DNA contamination from one experiment (N) to subsequent ones (N+1). In the first experiment, uracil is incorporated into contaminants through the use of ~1/3rd dUTP: ~ 2/3rd dTTP in the amplification reaction -- amplicons so derived contain a mixture of T and U bases. In the subsequent (N+1) experiment, UDG is added to the input sample prior to amplification. The UDG specifically cleaves uracil-containing contaminants inadvertently carried over from the first (N) experiment at room temperature. Upon elevation of the reaction to ~65°C, the UDG is heat-inactivated ensuring that only the target RNA (or 100% thymine-containing DNA) target is amplified.*

04.03.02 External positive controls

Beyond target DNA to test the LAMP primer amplification, the simplest positive control is the isolated nucleic acid itself, such as synthetic viral RNAs (e.g IDT, Twist Biosciences or from https://www.beiresources.org/) or RNA transcribed *in vitro* with a T7 promoter in the appropriate primer context (e.g. for the N gene; Zhang et al. 2020a). Such naked RNA can control for reverse transcriptase activity and subsequent DNA amplification but, since they are already purified nucleic acid, they do not allow for testing extraction efficiencies and cannot readily mimic methods designed to be used with 'direct' or 'extraction-free' methods as applied to e.g. saliva. Encapsulated viral particles are available, albeit at higher cost (e.g. Accuplex #0505-0126, Zeptometrix #NATFRC-6C, Cheng et al. 2020) and with the a greater chance of between lot-

and even within-lot variations. Consequently, some have abandoned their use in favor of internally benchmarked patient samples (by serial dilution, Bendesky, unpublished results and Vogels CBF, et al., 2021). Probably the most common sources of controls are heat-inactivated (NR-52286) or gamma-irradiated (NR-52287) cell lysates of VeroE6 kidney epithelial cells infected with SARS CoV-2 isolates available twice-yearly from BEI Resources.

04.03.03 Extraction positive control

An essential component of molecular diagnostic tests is an extraction or process positive control. This serves to ensure not only the proper functioning of the assay components (enzymes, primers, buffers, Mg^{2+}, dNTP), but also the successful extraction of nucleic acid from the sample of interest. A set of primers targeting DNA or mRNA expected to be in every sample regardless of infection status is used; for human diagnostic tests RNase P (e.g. Color Genomics, EUA) or beta-actin (e.g. Zhang et al. 2020a) are most commonly used. Designing primers across an exon-exon junction — a feature present only in RNA, not genomic DNA — would additionally confirm activity of the reverse transcriptase for robust RT-LAMP assays and is preferable but not essential.

Incorporating such internal control provides greater confidence for a true negative result, in that one knows that sample material was efficiently extracted, nucleic acid was transferred to the LAMP reaction and that amplification occurred. During clinical testing, a failed internal control invalidates the test such that no result is reported to the sample provider. During basic research or method development, a negative result suggests that one or more essential components of the reaction were omitted and/or that they have lost functional activity.

Extraction controls are either external (separate reaction from the clinical sample with SARS-CoV-2 primers, like MS2) or internal (RNaseP or actin amplification duplexed with SARS-CoV-2 primers). As with RT-qPCR tests, the latter is obviously preferable if two-color fluorescence detection can be achieved with sequence-specific methods (Section 05.02). However, the two component two reactions should be kinetically balanced so that one reaction does not dominate and consume all the limiting reagents (e.g. dNTPs, polymerase). If not optimized, sensitivity for SARS-CoV-2 will be impacted while conversely a strongly positive sample for SARS-CoV-2 could erroneously appear negative in the internal RnaseP/actin control. The former situation can be readily avoided by reducing the RnaseP/actin primer concentrations or omitting the loop primer(s) to slow this reaction despite the presence of high DNA/cDNA template concentrations.

Amplicon Detection

The very large amount of amplicon generated in LAMP reactions (a consequence of very efficient amplification) leads to more detection options. The fluorescent methods are akin to those used in RT-qPCR whereas others reflect changes to the bulk concentrations of reaction components (notably Mg^{2+}, PP_i and H^+), leading to a plethora of different detection methods for LAMP (Becherer et al. 2020). We focus on those most commonly used, which generate an optical (rather than e.g. electrochemical) signal or which rely on sequencing of amplicons. Two important distinctions between common detection methods include end-point vs real-time detection (**Figure 7A/B**) and sequence-dependent vs sequence independent methods (**Figure 7C**) (Zhang et al. 2014, Becherer et al. 2020). End-point tests are measured at a defined timepoint (e.g t = 30 min) whereas real-time tests follow the progress curves kinetically during the amplification process yielding the LAMP equivalent of a qPCR Ct-value. The latter is clearly more information-rich and so useful during method development or if semi-quantification is sought (LAMP is not generally used for absolute quantification) but it does impose the need for at least a simple fluorescence reader even if it is a smartphone camera combined with a light diode and optical filters (Section 06.03).

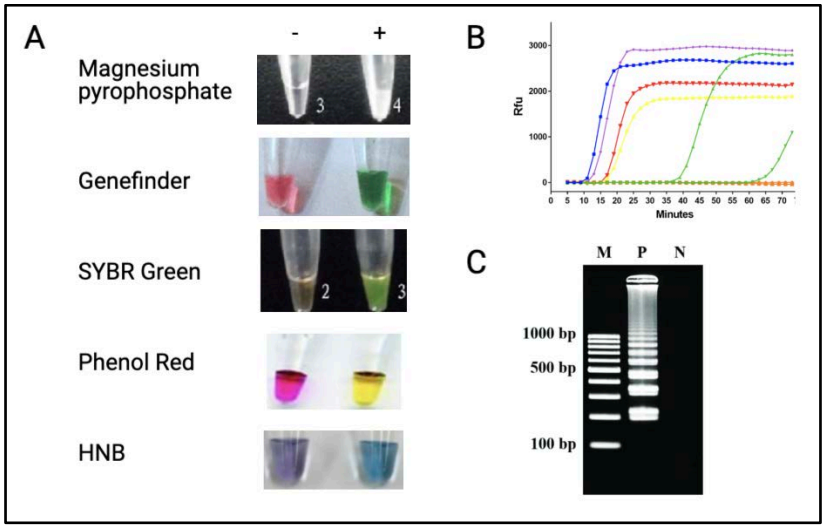


Figure 7. LAMP detection methods overview*. (**A**) Visual end point readouts use dyes that exhibit simple colour or turbidimetric changes upon amplification. (**B**) Similar to qPCR, real time detection methods use fluorescent dyes to monitor the increase in viral load as the amplification progresses. The fluorescent signal can be sequence-independent (e.g. DNA intercalating, 05.01) or sequence-dependent (hybridisation-based, 05.02). (**C**) LAMP products can, in principle, be verified by agarose gel electrophoresis followed by DNA staining although this requires post-amplification manipulation and the corresponding very real risk of between-experiment cross-contamination.*

LAMP amplification products can be detected by running a portion of the finished reaction through an agarose gel along with a DNA-staining dye or fragment analyzer (Agilent

Bioanalyzer, TapeStation or equivalent). Amplification leads to production of a distinctive ladder-like pattern. However, routine handling positive LAMP products on the bench is not advisable due to the very considerable quantity of DNA produced (10–20 µg vs 0.1-1 µg for PCR, Mori et al. 2001). Consequently, detection methods requiring opening reaction tubes post-amplification (e.g. Zhang et al. 2021) are increasingly less common and should be avoided unless extreme measures are taken to avoid contamination (Section 05.02.05) and which should almost certainly include the dUTP/UDG system (Kim et al. 2016; Section 04.03.01). Since many sequence-independent and sequence-dependent 'closed tube' detection methods have been developed, we suggest that these should be the default to avoid the very real (and very frustrating) risk of cross contamination and hence false positives.

05.01 Sequence-independent detection of RT-LAMP amplification products

Below we consider detection methods that rely on bulk changes to the concentrations of substrates or products, but which are not dependent upon the nucleotide sequence of the amplicon being created (*c.f* 05.02) (**Figure 8**).

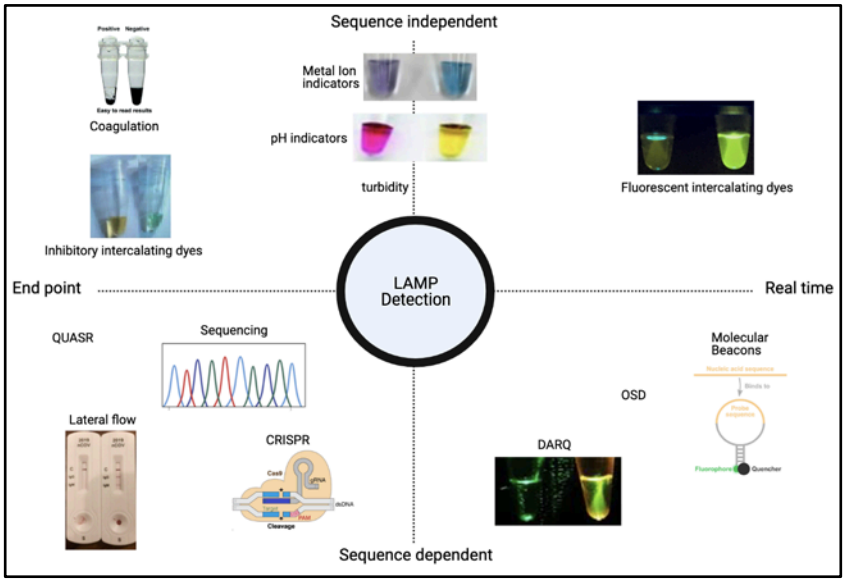


Figure 8 Categorization of Detection Methods. Reactions *can be monitored using simpler but less specific sequence independent methods (e.g pH changes) or the somewhat more complex but equally more specific sequence-dependent methods. These in turn can either be monitored in real time (e.g. DARQ, intercalating dyes) allowing amplicon formation to be monitored kinetically or as an end-point, stopping/recording the result at a defined time (e.g. QUASR). DNA sequencing is the ultimate sequence-dependent end-point method. When sequence-independent methods are used false positive results can be problematic. Most sequence dependent methods also allow for multiplexing multiple targets in the same reaction. Sequencing of amplicons can allow detection of different variants. Variations on these themes have been described -- the location of the icon in the 4-box is purely indicative.*

Turbidimetry: Historically, LAMP reactions were monitored in real time using turbidimetry arising from formation of a white magnesium pyrophosphate precipitate that correlates with the amount of DNA generated (Mori, 2001). Some disadvantages of this method (Zhang et al. 2014) include a low signal to noise ratio (improved by adding Calcium (Almasi et al. 2012)), the relatively low abundance of real-time turbidimeters, and incompatibility with minimally processed turbid samples. Turbidimetry was used in an early SARS-CoV-2 paper (Yan et al. 2020) along with the fluorescent metal indicator calcein (Tomita et al. 2008) which can also be detected by eye (light orange to light green) or under a handheld UV lamp. In general, turbidimetry has been relatively infrequently used for SARS-CoV-2 LAMP assays.

pH-based dyes: Originally developed by NEB and used in early SARS-CoV2 LAMP papers (Zhang et al. 2020a,b and Butler et al. 2020) this widely-used method is based on the color change of the simple pH-sensitive dye phenol red in a minimally buffered reaction as protons are released upon dNTP incorporation. In idealized conditions, the difference between a positive and negative result is visually striking (pink/yellow), but can be harder to determine for actual patient samples by eye due to intermediate or ambiguous color changes (Ben-assa et al. 2020; Huang et al. 2020, Coehlo et al. 2021). Additionally, the time window between the color change in positive reactions and negative controls can be small (Thi et al. 2020 ; Fowler et al. 2020), so close monitoring of incubation time is required. Since the reaction is minimally buffered, the pH of the input clinical sample can have a measurable effect on the colorimetric signal independent of (and appearing as) DNA amplification. This is particularly problematic when *minimally-processed* samples are combined with *minimally-buffered* reactions and is seen most acutely with saliva, although the effect is reported with some viral transport media. Some of these shortcomings can be overcome if reactions are followed in real time using a plate reader and data are analyzed using either derivatives or difference of measurements in 2 separate channels (Thi et al. 2020), but that can increase complexity for testing at scale or for point of care testing in resource-limited settings.

To address the color discrimination challenges associated with phenol red, Brown et al. 2020 synthesized two pH-sensitive dyes - LAMPShade Violet (LSV) and LAMPShade Magenta (LSM) - that respectively offer a purple/clear and pink/clear color change to distinguish between negative and positive results (Brown et al. 2020). LSV is reported to have fewer intermediate products and greater contrast relative to phenol red in similar minimally buffered reaction conditions (Yu et al. 2021 ; Brown et al. 2020).

pH-independent colorimetric dyes: Leuco crystal violet (LCV) (Miyamoto et al. 2015) turns from colorless to violet in the presence of double stranded DNA. Similarly hydroxynaphthol blue (HNB) (or eriochrome Black T) turns from violet to blue when free magnesium is removed due to formation of $Mg.PP_i$ (Goto et al. 2009 - there is also a change in fluorescence 540nm/610 nm,

Seok et al. 2016). These dyes generate relatively weak color changes, but can be amplified with algorithmic image transformations (Scott et al. 2020; Kellner et al. 2020; Martineau et al. 2017, see also 06.04). Both were recently used for the colorimetric detection of SARS-CoV-2 (Park et al. 2020; El-Toloth et al. 2020; Kellner et al. 2020; Lau et al. 2020).

Yet another type of colorimetric detection method was recently developed that involves the use of spermine, silica and charcoal (Mason and Botella, 2019). Spermine destabilizes amplified DNA, which in turn causes rapid flocculation of suspended particles of silica and charcoal. This is a low-cost method with results (gray/clear) that can be read via the naked eye but to date has only been applied to detecting DNA targets (Mason and Botella, 2020).

Intercalating fluorescence: More classically fluorescent DNA intercalating dyes have been used for both real time and end point detection. Dyes can differ in respect to their optical properties, signal-to-noise ratio, optimal concentration (which may also change depending on the fluorescence detection instrument being used), and also their inhibitory effects on the LAMP reactions (Quyen et al. 2019; Oscorbin et al. 2016; Seyrig et al. 2015). SYTO 9 for green fluorescence and SYTO 82 for orange fluorescence were found to be least inhibiting and have the best signal to noise ratios in multiple studies, but more common dyes such as SYBR Safe (Carter et al. 2017), EvaGreen (Diego et al. 2020; Diego et al. 2021), and even Ethidium bromide have been successfully used (Nagamine et al. 2002; Almasi et al. 2012).

Two additional dyes which have also been reported for SARS-CoV-2 detection, and which are both colorimetric and fluorescent include the Genefinder™ dye (Yu et al. 2020) and SYBR Green I (Champigneux et al. 2020; Lamb et al. 2020; Diego et al. 2020). Unfortunately, SYBR Green I is inhibitory to LAMP so must be added after the reaction has completed, this can be solved without opening tubes by wax encapsulation (Zhang et al. 2013), in contrast, the GeneFinder dye can be added before the reaction (Alamasi et al. 2015) but is not yet widely available outside of China.

Bioluminescence: The Bioluminescence Real Time (BART) assay (Kiddle et al. 2012) relies on bioluminescence produced when inorganic pyrophosphate release is coupled enzymatically first to ATP production via ATP sulfurylase and then to light formation via the luciferase/luciferin reaction. Although less widely adopted, BART has demonstrated high sensitivity (down to single copy), amenability to field detection (Hardinge et al. 2018), and has recently been demonstrated for SARS-CoV-2 detection (Fei et al. 2021)

05.02 Sequence-dependent detection of RT-LAMP amplification products

In contrast to sequence-independent detection methods outlined above, sequence-dependent methods generate a signal that is exclusively (or heavily) dependent upon the specific nucleotide sequence of the amplicon. With assays based on optical, magnetic, piezoelectric, electrochemical

and magnetoresistive sensing (Becherer et al. 2020), we focus exclusively on those creating a fluorescent signal (**Figure 9**).

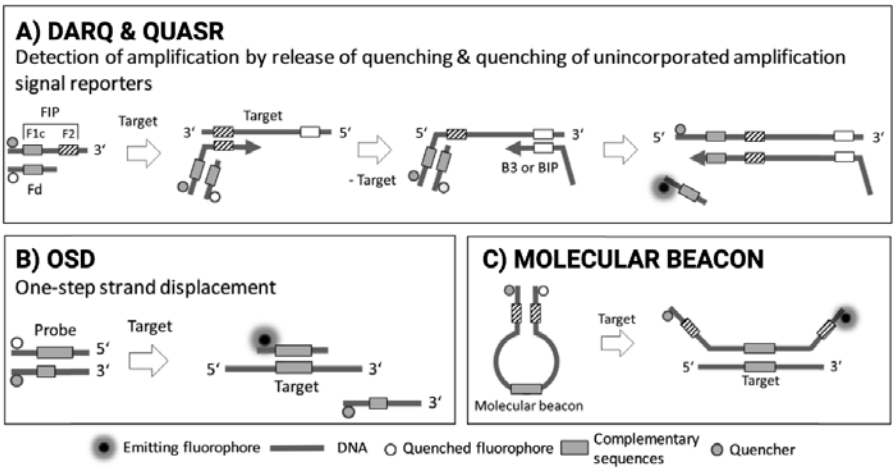


Figure 9. Schematic illustrations of some sequence-dependent fluorescent detection methods. *THE (A) DARQ, QUASR, (B) OSS and (C) molecular beacon methods can improve specificity of detection and processing for viral and other genome targets.*

In all LAMP assays, amplification of non-specific products such as primer-dimers can be common due to the high primer concentrations needed and the processivity of the polymerases -- this is an Achilles Heel of the methodology. Since sequence-dependent detection methods, in principle, will avoid detection of non-specific products, these methods are anticipated to be associated with fewer false positive results (Moehling et al. 2021). Another advantage of sequence-dependent detection is that two or more targets can be detected in one reaction tube via multiplexing of primer sets with different colored detection probes. This enables internal (within reaction) process controls using primer and probe sets for RNaseP/actin in addition to SARS-CoV2 genes, and/or allows detection of viruses causing symptoms similar to SARS-CoV-2 such as influenza (e.g. Zhang et al. 2020c).

The majority of sequence-specific reporting methods utilize a variation of a fluorophore-quencher duplex or hemiduplex probe. Signal results from the incorporation of part of the probe into the amplification product, which eliminates fluorescence resonance energy transfer (FRET) between the fluorophore and quencher to produce a fluorescent signal. Six such methods are described below, of which Molecular Beacons and CRISPR/Cas reporting require design of more bioinformatically complex probes or Cas protein/guideRNA complexes. In contrast, QUASR, DARQ, DP and OSD can be more readily developed, based upon existing RT-LAMP primer sets. QUASR reporting generates a bright fluorescent visual signal in comparison to DARQ and potentially has less inhibition of the reaction due to the shorter length of the quencher oligonucleotide. OSD has an added advantage in comparison to QUASR of further discriminating against false positives, due to the toehold strand displacement of its hemiduplex

reporter and the potential for discriminating variants. An important distinction is that while DARQ, DP, MolBeac and OSD enable real-time kinetic monitoring, QUASR is inherently an end-point measurement.

***05.02.01 QUASR* (Quenching of Unincorporated Amplicon Signal Reporters)** Ball et al. (2016) reported an end-point fluorescent method which stands out because it can be readily applied to an already established LAMP primer set for sequence-specific reporting. The modification involves adding a fluorophore on the 5' end of internal (FIP/BIP) or accelerating (LF/LB) primers. An additional oligonucleotide, complementary to but shorter than the fluorescently-labelled primer is added with a quencher on its 3' end. The T_m of the fluorophore-quencher oligonucleotide complex is designed to be at least 10°C lower than the LAMP reaction incubation temperature (~65°C) allowing for the incorporation of the fluorophore tagged primer into the amplicons generated by the reaction (see Supplemental Information S2). At the end of the assay, the reaction is cooled and unincorporated fluorophore-tagged primers anneal to the quencher oligonucleotide resulting, preventing fluorescence. However, if fluorophore-primers are incorporated into the amplicons they are protected from annealing to the quencher-oligonucleotide, and positive tubes will fluoresce. Thus far two previously published LAMP primer sets, NA (Zhang) and NM (Mammoth) have been successfully modified to use QUASR reporting (Bektas et al. 2021, Aidelberg & Aronoff, 2020).

***05.02.02 DARQ* (Detection of Amplification by Releasing of Quenching)** Tanner et al. (2012, 2018) describe a method similar to QUASR (see Moehling et al. 2021), in which the locations of the fluorophore and quencher on the primers/quenchers are reversed. The main functional distinction is that the quencher oligonucleotide is released from the FIP/BIP primer by way of the strand displacement activity intrinsic to *Bst* and related enzymes and this enables a continuous real time readout (*c.f* QUASR). DARQ has been employed in detecting SARS-CoV-2 as well as shown to be compatible with multiplex detection of Influenza A and B and a human RNA control (Zhang and Tanner, 2020). DARQ, similar to OSD, allows for real-time monitoring of amplification but has been suggested to suffer from inhibition owing to the full length complementary quencher sequence as well as less bright signal in comparison to QUASR (Ball et al. 2016). The OMEGA Amplification system from Atila Biosystems (Palo Alto, CA), the first company to receive an Emergency Use Authorization for RT-LAMP based SARS-CoV-2 diagnostics in April 2020, also uses the strand displacement activity of *Bst* to produce a sequence specific fluorescent signal (https://atilabiosystems.com/).

05.02.03 *One-step Strand Displacement (OSD)* Jiang et al. (2015) used a fluorophore/quencher oligonucleotide hemiduplex with a 10-11 base toehold on the fluorophore modified strand. This allows for competitive strand displacement as the amplicon is generated leading to a continuous fluorescent signal as the hemiduplex is disrupted by thermodynamic competition. Although this

presents an added constraint on primer design, and the OSD hemiduplex reporter must be prepared in advance, OSD has been shown to be especially robust in SNP detection using LAMP. OSD has been employed in the detection of SARS-CoV-2 successfully (Bhadra et al. 2020) (Maranhao et al. 2020). Another method, very similar to OSD, and employing the strand displacement activity of *Bst*, is termed Displaceable Probe (DP) RT-LAMP and has been used for the detection of SARS-CoV-2 alongside the multiplex detection of an internal control RNase P target (Yaren et al. 2020; see also a similar methodology described by Jang et al. 2021).

05.02.04: Molecular Beacons (MB)*:* Molecular Beacons have long been used as a probe in real-time PCR reactions (Tyagi and Kramer, 1996). They have a hairpin structure where the loop is complementary to the sequence being probed while the complementary stem sequences have a fluorophore and quencher attached. Upon annealing to the target, the complementary sequences in the stem of the molecular beacon are separated ending FRET between the fluorophore and quencher and resulting in fluorescence. The melt curves for specific- and nonspecific amplification using Molecular Beacons is also very specific to their targets (Sherrill-Mix et al. 2020). Unlike other sequence-specific methods that adapt existing primers, use of molecular beacons necessitate the design of a unique probe which, while relatively cheap when purchased at scale, can add significant cost, time and troubleshooting during method development.

Molecular beacons have the key benefit of targeting sequences that are not present in any of the primer sequences, e.g. in the loop region of the amplicon, and thus can be even more resistant to the detection of non-specific amplification. But this benefit, shared by OSD, also presents a challenge in finding a suitable sequence that provides stable hybridization at temperatures used for LAMP reactions. Previous studies have employed molecular beacons containing locked nucleic acids (LNA) to achieve annealing at LAMP temperatures and allow multiplexed detection of multiple SARS-CoV-2 and human control amplicons (Sherrill-Mix et al. 2020) and more recently of SARS-CoV2 variants (Sherrill-Mix et al. 2021). The very significant potential for molecular beacons as specific probes for LAMP is clear; the challenge will be to enable their comparatively easy, rapid and cost-effective design.

05.02.05: CRISPR-Cas cleavage systems. Emerging more recently, they have also been employed as a reporting mechanism for isothermal amplification products arising from LAMP (van Dongen et al. 2021) (Figure 10). First demonstrated as an endpoint detection platform for Recombinase Polymerase Amplification (RPA), ("SHERLOCK", Gootenberg et al. 2017) Cas proteins are coupled with a guide RNA specific to the genomic region being amplified. Molecular recognition of the CRISPR-Cas complex activates collateral ssDNAase or ssRNase activity that can be readily measured using either a quenched fluorescence reporter or biotin-FAM labeled probe if a lateral flow dipstick method is to be employed. This method, coupled with RT-LAMP rather than RPA, was commercialized by Sherlock Biosciences and received an EUA from the FDA in May 2020. This general schema has been further applied to SARS-CoV-

2 detection in what has been dubbed DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter) (Broughton et al. 2020) with several other systems being developed in parallel (e.g. Guo et al. 2020; Agrawal et al. 2020 ; Garcia-Venzor et al. 2021; see **Figure 10** and Table 1).

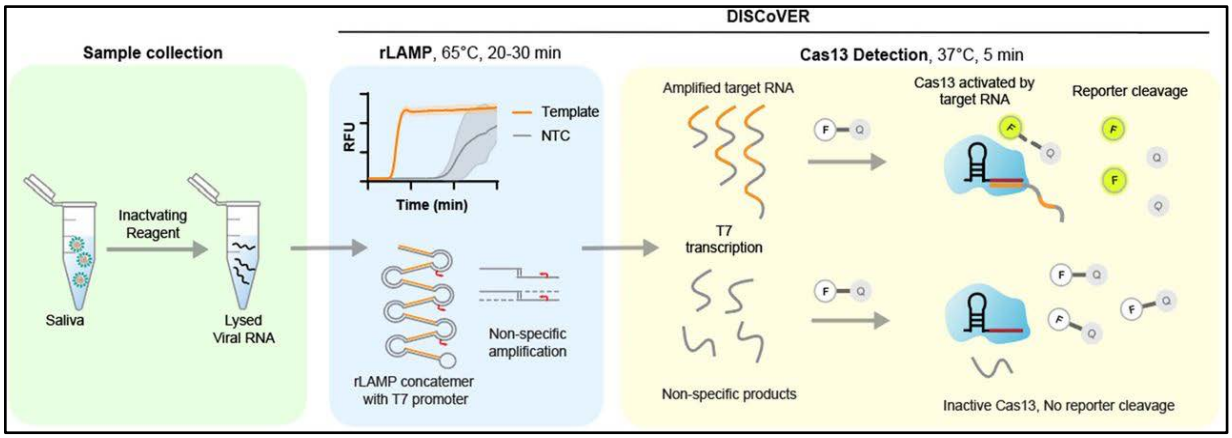


Figure 10: Schematic of the DISCoVER LAMP-CRISPR-Cas(Agrawal et al. 2020). *Viral RNA is reverse transcribed and amplified via LAMP then converted back to RNA using T7 polymerase. Cas13 enzymes are programmed with a guide RNA to specifically recognize the desired RNA molecules over non-specifically amplified products. Subsequent activation of Cas13 ribonuclease activity results in cleavage of quenched fluorescence reporter molecules. CRISPR-Cas provides additional layers of specificity and sensitivity albeit at increased cost and complexity.*

CRISPR-Cas based methods have undoubted potential and several key advantages such as increased specificity (via the gRNA) or potential for robust amplification-free Cas-based diagnostics using only the intrinsic collateral ribonuclease activity of the Cas enzyme (Joung et al. 2020). And yet, in their most commonly deployed formats they too suffer from a number of limitations in addition to increased complexity and cost per assay. Similar to Molecular Beacon-facilitated readouts, the already stringent primer design necessary for LAMP's particular amplification dynamics is further limited due to the requirement of suitable protospacer adjacent motif sites for the Cas enzyme-guide RNA complex. Furthermore, the Cas-gRNA must be added to the reaction after the RT-LAMP reaction is finalized to not interfere with the LAMP amplification. This can require opening of tubes post-amplification to add the Cas reagents and hence is associated with risk of contamination.

Two groups have attempted to solve this problem either by designing custom caps for reaction tubes which hold the Cas/gRNA complex for post-amplification cleavage of the ssDNA reporter while keeping it separate from the RT-LAMP reaction (Wu et al. 2020) or by simply adding the Cas/gRNA reagent to the cap of a standard tube to be mixed in at the end of amplification (Pang et al. 2020, Wang et al. 2020). Alternatively, a self-contained cartridge from Lotus that houses

the test strip and amplification tube makes a functional closed-tube lateral flow system, albeit at increased cost (adds ~$5/test). In a similar vein, Reboud et al. (2019) used a paper-based microfluidic technology for on field diagnostic, though they highlight the difficulties in post-amplification flow of products onto the test strip as a major challenge. As with Molecular Beacons, current results show very promising future potential, but the design and experimental methods for both need to be simplified to enable robust use at the benchside in a variety of settings and deployments (MacKay MJ, et al., 2020).

05.03 Sequencing Approaches Using RT-LAMP Amplicons

Since high throughput multiplexing and sequence verification of samples is particularly important for many applications (Rendeiro AF, *et al*., 2021), the use of both next generation high throughput sequencing (Illumina and Oxford Nanopore Technologies, ONT) and traditional Sanger sequencing are necessary (. This combination enables the analysis of thousands of samples in a single analysis run and provides detection by counting and sequencing using short read and long read sequencing platforms such as Illumina or Oxford Nanopore, respectively. Sanger sequencing in contrast allows low throughput sequence verification to confirm the molecular identity of LAMP positive samples. While Sanger sequence verification is less commonly used in routine testing (notably it suffers from the need to open a tube with amplified DNA), it may be required for some applications on a case-by-case basis.

Sequencing methods are increasingly important for detection of variants and mutations in the viral genome (Topic Box C, 10.16) which can provide a phylogenetic map of the epidemic (Butler et al. 2020), are useful downstream of the LAMP test as a secondary validation of the results, and for correcting false positives, informing on new strains of the virus and as an aid in contact tracing (Bull et al. 2020). However, they rely on more expensive (>$80K) instrumentation, specialized sample preparation, and a computational expertise typically found only in an advanced genomics laboratory. Thus, whereas many of the methods described in this review aim to meet the spirit of the REASSURED criteria, LAMP linked to NGS largely seeks to fill a distinctly different and complementary niche (Table 5).

05.03.01 Next Generation Sequencing (NGS) for LAMP

The ability to sequence LAMP amplification products prevents false positives due to non-specific amplification, since the complete sequence of each amplicon can unambiguously distinguish between the target gene and other mis-priming events. Thus, if not for the high cost and long turnaround times incurred, it would be desirable to apply NGS to LAMP amplicons. By analogy with PCR, one method to increase throughput is by multiplexing many samples in one sequencing run (Yelagandula et al. 2020). By designing a clinical sample-specific DNA barcode sequence that is either part of each primer set (Schmid-Burgk et al. 2020; James et al. 2020) or ligated to amplicons after amplification (Thi et al. 2020), amplicons from multiple samples can be pooled after being amplified in isolated LAMP reactions. Once the pool is

sequenced, the barcode of each amplicon is used to associate it back to its clinical sample of origin, while the genomic sequence of the amplicon is used to indicate whether the sample contains the target virus. This multiplexed approach harnesses the rapidly growing scalability of NGS platforms to drive the average cost per sample well below that of the upstream LAMP reaction itself. Nonetheless, risk/benefit of acquiring the short sequences from these complex concatemeric amplicons is high, and generally sequence analyses to follow evolving variants is done by applying the Arctic protocol for Illumina or Nanopore sequencing (more below) for at least 10x coverage.

While the sequencing time per sample is low, due to the high number of samples pooled in one run, the turnaround time can often be of the order of days. An exception to this is sequencing using Nanopore which can take as little as 1 hour when samples are not multiplexed at high numbers (James et al. 2020). For example, Parker et al. (2020) describe purifying the amplified LAMP DNA using a traditional column-based method and synthesizing a single rapid Nanopore library with RAD004 or a multiplex rapid library with RBK004. This technique is similar to RT-LAMP Sequencing devised by Thi et. al. (2020) that multiplexes many RT-LAMP samples after an intermediate PCR step. Since both techniques use rapid tagmentation -- a method that combines Transposase-assisted fragmentation of the target DNA with adaptor labelling -- they benefit from speed and can be sequenced using either a standard nanopore flow cell or an Illumina MiSeq.

05.03.02 LamPORE-Nanopore Sequencing

LamPORE from ONT combines LAMP with extremely portable and rapid nanopore sequencing (McIntyre ABR et al., 2016 and 2019). and thus has some parallels with the corresponding RT-qPCR-NGS method, SARSeq (Yelagandula et al. 2020). This technique involves a simple library preparation that adds unique molecular barcodes (UMI) to each sample and sequencing using a standard Oxford Nanopore flow cell, allowing for large numbers of samples to be multiplexed and analyzed simultaneously (Ptasinska et al. 2020). For example, using 12 different LAMP barcodes combined with 96 rapid nanopore barcodes results in 1,152 multiplexed samples. With nanopore sequencing for 4 hours on either a MinION or GridIon sequencer to generate sufficient coverage for each sample (James et al. 2020), it has a theoretical maximum capacity of 15,000 samples per 24 hours using the GridIon equipped with 5 flow cells allowing for scalability and high throughput with LIMS- compatible quality control reports. This high throughput approach has been successfully employed in a large-scale European study involving over 20,000 samples (Ptasinska et al. 2020). In a 3-week clinical validation study with 1200 participants (3,966 swab and 19,461 saliva samples) LamPORE showed a sensitivity of >99.5% using both swab and saliva samples from asymptomatic participants compared to a reference CE-IVD marked RT-qPCR assay. In the symptomatic cohort (incidence 13.4%), the sensitivity and specificity were 100% (Ptasinska et al. 2020).

Another advantage of LamPORE is the ability to amplify several targets simultaneously, from multiple genomes and/or from multiple regions of the same genome. Since the amplicons are sequenced, a multiplexed LamPORE reaction may detect a broad range of pathogens or strains distinguished by specific variants in a sample in a single sequencing run. Specifically, James et al. monitored three regions of the SARS-CoV-2 genome in addition to a beta-actin internal control (James et al. 2020), the sequencing counterpart of the multiplexed fluorescence assays described above (Section 04.03.03 and 05.03. e.g Yaren et al. 2020 ; Bektas et al. 2021; Zhang and Tanner, 2020) .

05.03.03 Verification of LAMP positive samples using Sanger and Nanopore Sequencing

Verification of positive RT-LAMP products can be accomplished using traditional molecular techniques such as the low throughput, low-cost method of Sanger sequencing (Parker et al. 2020). This technique involves DNA extraction of the LAMP positive sample using a column-based DNA extract (QIAamp DNA mini kit; Qiagen Hilden Germany), quantification using spectrofluorometry (Qubit Instrument Life Technologies, Carlsbad CA USA), followed by Sanger sequencing. Previous studies have indicated that 6ng of purified amplification product can be cycle sequenced in the presence of 10% GC Melt or DMSO using the Loop B or Loop F primer with the resulting sequences used in NCBI BLAST comparisons.

Infrastructure: Hardware, Software, Bioware

06.01 Introduction

A core strength of LAMP is its applicability to multiple settings, scales of testing, budgets and the extent of available infrastructure. It can be readily adapted to existing laboratory equipment, since the most basic components are a device that can maintain ~65^{o}C for ~30 minutes. This inherent simplicity has led to a slew of creative hardware solutions including a simple *sous vide* heater. Thus, isothermal LAMP hardware joins their low-cost PCR counterparts (e.g the 5-tube PocketPCR). Many open-source options described below cost between $400-$1,000 and are able to perform 8 reactions at a time thus lowering the cost to entry for LAMP.

However, for those with greater resources or a need for higher throughput, mid-priced commercial options offer greater technical support and come in more complete, user-friendly packages using 8- or 96/384-well reaction incubation and plate-reader technology. One (of many) example is the 384-well incubator-reader from BioTek Neo2 which enables both colorimetric (e.g. pH and Mg^{2+} sensing) and fluorogenic (DNA intercalation and sequence-specific probe) readouts. In principle, 1536-well screening with fluorogenic LAMP should be viable in 2-10µL reaction volumes if evaporation can be controlled.

06.02 Budget Breakdowns

A summary of different deployment models for LAMP testing is provided below, distinguished by throughput, cost, and resources needed (Table 3). Two important notes. Firstly, we quote prices as if the items were purchased in mainland USA (i.e these are the *lowest* reference prices). Secondly, the descriptions/builds below are purely exemplary, being one configuration used by a US laboratory; alternatives could be substituted based on individual budgets and circumstances.

Tier 1: "Basic LAMP": This tier is suitable for those interested in piloting the use of LAMP with the minimum of investment or with an intended throughput of <100 tests per day, for example using individual PCR tubes or 8-well strip tubes. The equipment will be available in most biological laboratories and many high school science classrooms. The main cost is the LAMP test itself (~$8 per test, USA if purchased as a preformulated kit).

Tier 2: Moderate Throughput LAMP: Expands LAMP capabilities, moves to 96-well plate formats for parallel temperature-controlled incubation while the plate reader allows for quantitation of the signal over and above reporting results visually as a color change. This system has the capacity for up to ~1000 tests per day depending on staffing.

Tier 3 High Throughput RT-LAMP: The solution described here expands LAMP capabilities beyond 1000 tests per day by automation of many of the core processes using benchtop (vs. integrated) automation systems. These solutions would be found in e.g Pathology laboratories or centralised testing facilities. Since they likely fall within a Regulated realm, testing using such systems also have Medical Device, Regulatory and personal data privacy/security consequences. In a research setting, the Mason lab received Institutional Board Review (IRB) approval for the use of a colorimetric LAMP kit (NEB) with the TINY testing device (Snodgrass et al. 2018), the results from which were automatically and securely uploaded to a local Pathology department for review and potential follow up with a certified SARS-CoV2 test (Section 7).

	Item	Example (part number)	Cost (USA)
L	Pipette tips, nitrile gloves, biohazard bag, Lo-bind 1.5mL microfuge tubes PCR plate or 0.2mL tubes	76322-160 89428-750 10035-976 80077-230 10049-108	~ $1000
L	Manual P100 Pipette	76335-742	~$300
L	SARS-CoV-2 LAMP kit (100-500 rxn)	e.g NEB E2019S (Colorimetric) e.g. OptiGene RT-LAMP KIT-500	~ $8/test
L	Sous vide/water (65°C heat source)	~ $40	~ $40
M	Thermocycler (tube/plate)	71003-564	~ $9K
M	Biosafety cabinet	89260-050	~ $11K
M	Precision Convection Oven	PR305220M	~ $3K
M	Plate Reader	e.g. Biotek Neo 2	~ $36K
M	Corning LSE Vortex Mixer	6775	~ $200
M	Corning LSE Mini Microfuge	6770	~ $200
H	Refrigerated Centrifuge	5942000245	~ $17K
H	HTP plate reader	E.g BioTek Neo2S	~$43K
H	PX1 PCR Plate Sealer	1814000	~$4.5K

H	Xpeel plate seal remover	1150L21	~ $37K
H	BioTek™ MultiFlo FX MultiMode Dispenser	BTMFXP1	~ $18K
H	Hamilton Starlet liquid handling robot (LHR)		~$145K
H	Chemagic 360 (automated magnetic-bead based nucleic acid extraction)	2024-0020	~$130K

Table 3*: Deployment models for LAMP testing. The hardware infrastructure required for different scales of testing are indicated by progressive increases in the capital infrastructure needed. In general, testing moves from tubes to plates and from manual pipetting/reading to automated processes. The items (and their approximate prices) are only intended to be exemplary and indicative, being taken from one US-based genomics research laboratory.*

06.03 Hardware designed for LAMP-based assays

The most common hardware used for RT-LAMP is a preexisting qPCR reader or conventional plate readers capable of 65ºC incubation (e.g. Biotek Neo2, Molecular Devices SpectraMAX M65C, or Perkin Elmer Nivo). More simply, a wet/dry heating bath, or block, can be combined with either visual colorimetric detection (e.g phenol red) or simple LED fluorescence excitation with smartphone data capture (e.g SYTO9). In this section, we exemplify -- but do not necessarily endorse -- some of the now many mid-priced hardware solutions available to LAMP researchers with references for use with SARS-CoV-2 where available.

06.03.01 Optigene: Genie

The Optigene© Genie II or III performs real time fluorescence-based LAMP providing quantification of the progress curve in addition to melt curve analysis of the products as an aid to quality control (template-specific amplicons often have a characteristic melt temperature). Devices cost between $3,000 and $15,000 and are capable of processing 8 or 16 reactions at a time. A recent example of its use was for a large saliva-based clinical study investigating use of chelex-100 during the preanalytical processing step (Howson et al. 2021)

06.03.02 Eiken: Simprova

Eiken Chemical Co., Ltd. has developed an automated cartridge-based system for sample processing/RNA extraction and molecular testing (functionally similar to the CEPHEID GeneXpert PCR system). Using a variety of biospecimens, the Simprova system is claimed to have good or better RNA/DNA extraction performance compared to QIAGEN Mini Kits with a

shorter turnaround time (15 min vs 40 min). The multi-well testing chip (one input, 25 test reactions) contains dried LAMP reagents within a closed system to minimize cross-contamination and a fluorophore-guanine quench system for detection (Yonekawa et al. 2020). Although results using Simprova with influenza, respiratory syncytial virus and metapneumovirus have been published (Takayama et al. 2020) , we are unaware of SARS-CoV2 specific publications beyond the Eiken Loopamp kit approved in March 2020.

06.03.03 Qiagen: ESEQuant

The ESEQuant TS2 is a small fluorescence plate reader suitable for fluorescence-based LAMP that uses light-emitting diode and filter technology combined with a touchscreen display, bar codes, RFID reader and LIMS connectivity. With fluorescence measured in up to 6 channels it is well suited to multi-color, multiplexed readouts (see Section 5) while melt curve capabilities provide for quality control as described previously. Publications citing this reader appear limited at the time of writing.

06.03.04 GeneMe: Testing Cube

Designed for use in concert with the FRANKD test strips for SARS-CoV2, the Testing Cube is a small (2.5 kg), affordable (~$250 depending on country, off-the-shelf components), open-source diagnostic device (www.geneme.eu). The design specifications have been openly shared on hardware sites including Team OSV, GitLab, Welder and Wevolver. The Testing Cube consists of a single strip of eight wells (6 tests, 2 controls) to run the FRANKD (and other isothermal) tests for approximately 30 minutes before delivering the results.

06.03.05 AXXIN: T-8 Benchtop fluorometer

The AXXIN T8 reader is a small footprint, lower cost (retail $5000), 8-PCR tube, dual-wavelength incubator reader with a 10 sec scan read time. It has the ability to mix viscous liquids if a magnetic ball bearing is included in each tube (this feature was designed for the high viscosity solutions used for RPA). In this issue, Natoli et al. 2021, describe methods used to adapt the T8 for use with LAMP at 65^{o}C (vs. RPA at 41^{o}C), particularly the need to add mineral/paraffin oil to limit evaporation since the T8 does not have a heated lid.

06.03.06 Diagnetix: BioRanger

The BioRanger (diagenetix.com) is a handheld device to support LAMP to promote field-based agricultural diagnostics and their related "Assimilating Probe" sequence-based fluorescence technology (Kubota et al. 2011; Kubota and Jenkins, 2015). The 8-tube, two-wavelength device incorporates control with a programmed heating cycle. It is intended for low-resource field applications (~$3000) and interfaces wirelessly through Bluetooth to an Android app with a range of connectivity features. For use with thermally stable polymerases (Chander et al. 2014) the BioRanger can be programmed with a “lysis” step to promote release of nucleic acids prior

to user-defined amplification programming including an endpoint melting analysis to confirm amplicon purity. A colorimetric version of the instrument has been developed for use with e.g phenol red/pH changes (Diaz et al. 2021; submitted, this issue) with very promising results and times-to-positive that are shorter than when detected visually (N. Tanner, unpublished).

06.03.07 Biomeme: Franklin

The thermocycler device developed by Biomeme (the Franklin™) for qPCR can in principle also be used for isothermal measurements although we have found no reports of its use with RT-LAMP. Different models enable either 1, 2 or 3 wavelength detection in 9 tubes simultaneously when measured on an Android smartphone (cost $6,000-$10,000).

06.03.08 Prakash: Snap Dx

SnapDx (https://www.snapdx.org/), an off-shoot of the Prakash Lab at Staford, develops numerous relatively simply built portable devices for low resource or home-testing settings using saliva RT-LAMP. The core SnapDx product is currently (April 2021) undergoing trialing. Other devices developed include the $5 handyfuge, an open-source solution for low speed centrifugation steps in point of care RT-LAMP tests and being successfully applied to the Rabe-Cepko protocol (Rabe and Cepko, 2020).

06.03.09 Biopix-T: Pebble

The Pebble incubator-absorbance reader from Biopix-T (https://biopix-t.com) is a compact device for performing real-time colorimetric LAMP with 8 PCR tubes, operating via an Android application and connecting via Bluetooth to a smartphone (Papadakis et al. 2020).

06.03.10 Canon (Genelyzer FIII)

A portable battery-operated fluorescence-based incubator-reader platform for low infrastructure settings (https://jp.medical.canon/products/dnachip/genelyzer_F/index), the Genelyzer FIII was demonstrated to have good diagnostic performance with NPS-based RNA-extracted and, to a lesser extent, with extraction-free protocols (LOD 25 and 1400 copies/reaction respectively) using Canon one-enzyme isothermal mastermix and Orf1b primers (Yoshikawa et al. (2020).

06.04 Software

06.04.01 Primer Design Tools

Given the need to simultaneously design multiple primers which are predicted to bind specifically and uniquely to the target, the only reasonable path is to employ dedicated open-access software tools to help guide primer selection (Section 04.01). The most commonly used tools are listed below along with their developers:

- PrimerExplorer V5 (Fujitsu/Eiken)
- LAMP Primer Design Tool (NEB).
- OligoAnalyzer (IDT)
- LAVA: Lawrence Livermore National Lab (Torres et al. 2011)
- GLAPD: Shanghai: (Jia et al. 2019)
- Primer Analyzer (ThermoFisher)
- OligoCalc (Northwestern)

While many SARS-CoV2 primer sets have been published (Table 2, Supplementary Table S3, see also Janikova et al. 2021) these tools can be used for those interested in deeper exploration and can be an important hedge against "standardized" primers becoming less effective or biased as the virus evolves (Topic Box C, Section 10.16). Primer design performed well is non-trivial; while these *in silico* tools are both valuable and necessary, several selected sets should be tested experimentally, especially for cross-reactivity in multiplexed or combioplexed assays (Section 04.01)

06.04.02 Color Interpretation

06.04.02.01 Colorimetry.net (Mg/HNB-based assays)

https://colorimetry.net/about/ is a simple web service for automatic computer-assisted enhancement of images of color changes associated with use of Hydroxy Naphthol Blue (HNB) dyes as a measure of LAMP amplification. It's enhancement strategies seek to increase perceptual accuracy when performing a visual readout. The biochemical impetus for the work is the original publication of Goto et al. (2009) as exemplified by Kellner et al. (2020).

06.04.02.02 Phenol red (pH-based assays)

Colorimetric RT-LAMP assays using weakly buffered solutions and a pH indicator can at times lead to subjective and ambiguous results (neither a definitive red or yellow color). In one such assay, the differences in color amongst the positive and negative samples were identified through color decomposition and analysis in the color CIELab space (Gonzalez-Gonzalez et al. 2020). Yoo and colleagues have developed a tablet PC-based portable device (FlagMan) using three signal categories, DEF (decisive [main colour peak], effective [purity of color] and fuzzy [‘clarity]) which are akin to hue-saturation-value (HSV) coordinates (Yoo et al. 2020). McLaughlin (this issue) have similarly developed a software application for color assessment as described in Topic Box B (LAMPPlatereader.app).

06.04.02.03 Artificial intelligence LAMP (ai-LAMP)

Rohaim and colleagues combined automated image acquisition with the use of artificial intelligence to "engineer a novel hand-held smart diagnostic device." (ai-LAMP) applied to a colorimetric (pH, phenol red) readout. Collected images were converted from RGB color space

to YUV color space for use with a convolutional neural network (CNN), which has been shown effective in many contexts (Haibe-Kains B *et al*., 2020). Using ~200 suspected COVID-19 patients the platform was shown to be reliable, highly specific, reducing assay run-time and detection subjectivity (Rohaim et al. 2020).

06.05 Emerging technologies

The majority of SARS-CoV2 research using RT-LAMP uses comparatively well-established base technology. In this section we review examples of emerging technologies and methodologies, based often on adaptation of previous work with other viruses/pathogens. Some are relatively 'high tech' (e.g. Qin *et al*., 2020) while others are purposefully "low tech", being fit for a very different purpose.

06.05.01 Smartphone-enabled LAMP

Technological integration of the light/camera features of smartphones or tablets into testing devices has increasingly become popular, creating tube- and microfluidic sensory systems (Farshidfar N, Hamedani S. 2020. Nguyen et al. 2020). Building on the ability to receive and process data from other sensors and devices, their appeal lies in their comparatively low cost, widespread availability and their ability to connect medical professionals and patients. A second benefit of remote testing is to allow patients exhibiting mild respiratory symptoms to get tested without having to attend often highly overcrowded centralized healthcare facilities.

Several researchers have used smartphones/tablets for tube-based readouts (Yoo et al. 2020., Rohaim et al. 2020), predominantly for measurement at one wavelength. In contrast, while developing tests for West Nile (WNV) and chikungunya viruses (CHKV). Ball et al. (2016) developed the QUASR methodology (see Section 05.02) enabling dual-color duplexing of targets using a flashlight LED, red/green plastic lighting gel sheets, and detection with an dedicated app-enabled iPhone 6.

In addition to tubes/plates, smartphone detection is also used with microfluidic 'lab-on-a-chip' approaches (see below). Developed first in a non-clinical model system (Sun et al. 2020), Ganguli et al. (2020) combined a spatially-multiplexed microfluidic chip with a cradle to house blue LEDs and a filter/smartphone to detect the green fluorescence and were able to detect SARS-CoV2 RNA in simulated nasal samples.

06.05.02 Microfluidic platforms

The vast majority of devices use classical tube or plate formats. However, as in other fields (HIV: Safavieh et al. 2017; Zika: Roy et al. 2017 ; Song et al. 2016), some have explored assays performed in microfluidic devices, driven largely by their potential to enable closer-to-point-of-care testing and with pre-dried reagent cartridges lower molecular enzyme/reagent consumption

(Zhang et al. 2019; Berkenbrock et al. 2020; Farshidfar and Hamedani 2020; Augustine et al. 2020). While paper-based devices are formed by a series of hydrophilic cellulose fibers that move reaction liquids by capillary action *via* absorption, another class of channel-based devices are engineered through nanofabrication (Basiri et al. 2020; Rodriguez-Manzano2021).

06.05.02.01 Paper-based microfluidic devices

Paper-based microfluidic diagnostics emerged largely to enable very low cost, portable, disposable true point of care tests (Gong and Sinton, 2017, Carrell et al. 2019). Indeed, Seok et al. (2016) were able to detect meningitis DNA using a LAMP-based three-layer stacked paper microfluidic device. Using dried (not lyophilised) LAMP reagents, the sample is distributed into three test and one control zone via capillary action; amplification is monitored by changes in HNB **fluorescence** (c.f absorbance) ($\lambda_{ex/em}$ = 540nm/610nm) allowing detection of 10^2 - 10^5 DNA copies. Similarly, Varsha et al. (2020) describe an even simpler paper device for Leptospira DNA using commercial Whatman #1 paper, again with dried LAMP reagents detected via smartphone. Kaarj et al. (2018) used the wax-printed paper microfluidic as both a means for partial purification of Zika virus RNA (see Section 3) and also as a substrate for the smartphone-based colorimetric RT-LAMP reaction.

Interested readers are directed to Topic Box 10.18 which further expands on nucleic acid purification and microfluidic technologies under the "LAMP on Paper" umbrella. Despite this precedent and potential, only one paper-based RT-LAMP diagnostic device ('COVIDISC') for SARS-CoV-2 has been described (Garneret et al. 2021). Viral RNA is extracted onto a fiberglass membrane, dried, and then folded onto paper-based "discs" containing freeze-dried LAMP mixture and primers. The RNA is eluted onto the discs and the heating of the portable system enables fluorescence readout of the discs using QUASR (Section 05.02, Ball et al. (2016); Bektas et al. 2021).

06.05.02.02 Nanofabricated channel based-microfluidic devices

An early channel-based microfluidic devices for SARS-CoV2 included a disposable microfluidic polymer cartridge containing two luer-lock inlet ports (sample and RT-LAMP reagents), a 3D reagent mixing and serpentine heated region, this battery-powered device was able to detect SARS-CoV2 using a fluorescent dye/smartphone combination (Ganguli et al. 2020). Two microfluidic systems based on centrifugal liquid flow have been reported. With the objective of simplifying the sample processing step, Soares et al. (2020) developed an integrated smartphone-enabled centrifugal microfluidic platform for a sample-to-result fluorescence-based RT-LAMP test. Using modified agarose beads, their device enhances the signal specificity and mitigates the impact of collection media on weakly-buffered colorimetric LAMP assays (Section 03.05). de Oliveira et al. (2021) describe a polystyrene-toner (PS-T) centrifugal microfluidic device manually controlled by a fidget spinner. The amplification (5µL) was controlled with a thermoblock at 72°C for 10 min with automated on chip visual detection. Finally, an elegant but

significantly more complex solution uses electric field-driven microfluidics (isotachophoresis (ITP)) combined with CRISPR-based diagnostics to enable aspects of RNA purification from NPS, ionic focusing/concentration and detection in a single microfluidic device. (Ramachandran et al. 2020).

The path to commercial and clinical use for Lab-on-a-Chip' (and similar) devices has been long and not without its challenges; out of the lab to a scaled-up, robustly manufactured product with a simple procedure for routine use by non-experts. Certainly this is possible -- several such commercial systems exist e.g the Cepheid GeneXpert or the Eiken Simprova -- but these are currently the exception rather than the rule. We watch with interest to see whether the COVID pandemic provided the impetus for microfluidic devices to reach a tipping point where their potential for low cost mass-production will be realised.

06.05.03 Digital LAMP (dLAMP)

Digital amplification methods partition bulk samples into many small or localized compartments, so that target viral nucleic acid molecules are isolated in small volumes. As a result, molecules are amplified independently, generating a localized positive or negative signal that can be considered digital. Each signal is counted in an endpoint measurement and the sum over all positive compartments represents the total number of viral copies detected in the sample.

Although several implementations of dLAMP have been previously reported (Rolando et al. 2020; Yuan et al. 2020), none-to-date have been applied to detect SARS COV-2. Prior work has explored the dynamics and sensitivity of dLAMP using micron-sized chambers to compartmentalize the sample (Khorosheva et al. 2016, Rolando et al. 2019). Others have used droplet microfluidics, in which LAMP mixture is partitioned into micron scale drops in oil and fluorescence from the drops is detected using a simple optical system and camera (Hu et al. 2020) (Schuler et al. 2016). Partitioning can also occur within a gel matrix environment, limiting the diffusion of the LAMP-amplified amplicons and generating localized fluorescent spots that can be imaged using a smartphone with no additional optical magnification (Huang et al. 2018). Finally, dLAMP can be performed on a commercial membrane filter (Lin et al. 2019) in which the sample is partitioned into the pores while oil isolates the pores from each other. While these methods differ in their implementations, they all share the same advantages derived from the isolation of templates: 1.) absolute quantification of viral copies compared to real-time qPCR which requires a standard curve; 2.) template isolation which limits the effects of false positives due to contamination or non-specific amplification in the sample.

06.06 Solutions for use in Limited Resource Settings

Developing and deploying nucleic acid detection systems that have low capital cost, have little reliance on traditional medical infrastructure, are easy to fix or require no maintenance, can be transported between small rural clinics, and are easy enough to use without extensive training can significantly impact the delivery of diagnostic services in limited resource settings (Yager et al. (2008), Jani and Peter (2013), Urdea et al. (2006)). For example, it has been shown (Cox et al. 2015) that decentralization of diagnostic testing by implementing the GeneXpert PCR system was able to reduce the time for treatment of tuberculosis from 71 days to 8 days in Cape Town, SA. The relatively high cost of these established PCR systems and moderate infrastructure requirements however tend to limit their distribution beyond mid-scale facilities and limit their portability.

LAMP based tools have proven a popular method for addressing these challenges primarily because the single temperature amplification reduced the required complexity of the instrument while maintaining high diagnostic accuracy. Successful development and deployment of systems which can address the challenges mentioned above could catalyze the expansion of LAMP, particularly for use in remote, limited resource settings or as part of a much more distributed testing network.

06.06.01 Intermittent- or zero-electricity LAMP devices

Liao et al. 2016 created a portable electronics-free "Smart cup" performing LAMP on a microfluidic chip, with heating accomplished by coupling to an exothermic Mg–Fe alloy hand-warming pouch, ~$0.15) that enabled a relatively constant temperature of 68^{o}C to be maintained for an hour. With a fluorescence/smartphone system, they were able to perform 4 reactions in parallel, detecting HSV-2 DNA down to 100 copies/reaction. (Snodgrass et al. 2018) describe detection of Kaposi's Sarcoma Herpes Virus in human biopsy samples in Uganda with a portable device that can operate based on various live or stored energy sources including electricity, solar energy or a flame - performing 6 reactions in parallel. They demonstrated the system was robust against power interruptions and could be used with equivalent efficacy by trained lab personnel and local providers. The technology was based on earlier versions by Jiang et al. (2014) and Snodgrass et al. (2016) which used solar energy focused on a traditional microfluidic chip. Labarre et al. (2011), Singleton et al. (2014), Curtis et al. (2016) all demonstrate innovative small scale, typically single use, devices which do not require the use of external electricity to perform detection on a small set of devices. Stedtfeld et al. (2012) demonstrated an early system that uses mobile technology (iPod touch) and microfluidics to perform up to four parallel samples.

06.06.02 Battery operated and other low-cost systems and accessories

Velders et al. (2018) built and programmed a single-tube prototype of a portable, one-temperature, battery-operated open-source Arduino shield for LAMP with a fluorescence readout. With moulded polydimethylsiloxane (PDMS) to fit PCR tubes, heating was achieved via a nichrome wire and is designed to be straightforward to assemble. Priye et al.(2017) developed a LAMP box to detect arboviruses using a smartphone, which uses resistors and an aluminum plate as a heat source. This 8-reaction device costs ~$100 but requires specialized reaction vessels to work optimally. Using 3D printing, Gonzalez-Gonzalez et al. (2020) describes a simple circulating water holder for standard 0.2mL PCR tubes when coupled to phenol red detection. Priye et al. (2017) developed a LAMP box to detect arboviruses with resistors and an aluminum plate as a heat source, combining QUASR with chromaticity-based smartphone analysis. Although practical and comparatively cheap (~$100), the device requires specialized reaction vessels to work optimally. Some open hardware devices that could be adapted for LAMP temperature control include: PocketPCR from Gaudi labs (Pocket size USB powered PCR ThermoCycler and PocketPCR), NinjaPCR (for PCR and LAMP) and a 3D printed RT-qPCR device created for infectious disease diagnostics (Mulberry et al. 2017).

miniPCR's blueBox™ S or Pro Transilluminators with Imaging Hood can illuminate plates with a smartphone docking system ($250-$350) while the battery-operated P51™ Molecular Fluorescence Viewer can illuminate 8-tubes ($30), providing a low cost, manufactured solution. Similar readers can be built individually using open source methods (e.g. for education purposes, GMO Detective.com).

06.06.03 Lyophilization and Dry Storage of RT-LAMP Reagents:

A reliable supply chain and storage capacity is essential in all settings but is particularly problematic for regions with poorly developed infrastructure, notably those lacking cold chain or storage (2-8^{o}C or -20^{o}C). Dried and/or lyophilized reagents are components of several cartridge-based systems (TwistDx/Abbot for RPA, Eiken's Simprova for LAMP, Cepheid's GenExpert for PCR) and also components of some paper-based microfluidic devices (see above, Garneret et al. 2021, Varsha et al. 2020., Seok et al. 2017). Others have sought to prepare lyophilized (freeze-dried) reagents as standalone reagents with the objective of stabilizing and prolonging room temperature shelf-life (Pack et al. 2008, Challener 2017, Chen and Ching 2017). Using lyophilized and premixed reagents (e.g mastermix containing the required primers, detection dye etc) necessarily also simplifies and shortens assay preparation (Carter et al. . 2017).

While individual methods (temperatures, pressures for lyophilization), most methods contain D-trehalose as a protein stabilizer, avoid the use of hygroscopic glycerol-based stock solutions (Aidelberg & Aronoff, 2020; Xu et al. 2020), and often contain additional excipients (mannitol, BSA, and polyethylene glycol). Reagent stability under different conditions can be measured using an accelerated aging/Q_{10} method (Chua et al. . 2010).

Dried (not lyophilized) RT-LAMP master mixes for SARS-CoV2 containing primer sets ORF1ab, N5, or N15 and the amplification enzymes have been described (Diego et al. 2021) with a projected shelf-life (via the Q_{10} method) of 2 months at room temperature. Similarly, Yaren et al. (2020) first removed glycerol from commercial enzyme preparations before preparing lyophilized master mixes for their RT-LAMP assay.

06.07 Open Research Infrastructure

06.07.01 Open Science in Research: for COVID-19 and beyond.

The global urgency to contain the COVID-19 pandemic has led to large-scale global collaborative efforts between organizations, communities, and individuals from many different backgrounds (*e.g. gLAMP, JOGL, Access to COVID Tools Accelerator, Helpful Engineering, Open Source Medical Supplies, WHO COVID-19 Technology Access Pool, UN Technology Access Partnership*). To ensure knowledge flows efficiently and advances our understanding of the virus and how best to contain the pandemic, open access to COVID-related publications and data was encouraged (https://wellcome.org/coronavirus-covid-19/open-data). The Open COVID Pledge was established to enable holders of >600k patents to waive their IP for use addressing the impacts of the pandemic (Contreras et al. 2020). Throughout the pandemic, scientists from around the world have come together to develop and improve RT-LAMP assays for detecting SARS-CoV-2. Scientists in various regions and under distinct regulatory regimes have congregated within and across multiple consortia and working groups; the gLAMP community is one such group. Here and elsewhere various research outputs such as protocols have been shared e.g. via the protocols.io Coronavirus Method Development Community and multiple websites have been established by researchers to share know-how and protocols related to their tests (e.g. Saliva Direct). One such group is the Vienna COVID-19 Detection Initiative (VCDI), informally known as “the Vienna group”. Their most notable LAMP-related efforts include: development of colorimetric LAMP with HNB dyes, development of `bead-LAMP` featuring purification with magnetic beads, development of a gargle-based sampling (see Kellner et al. 2020), and in-house preparation of Bst-LF and HIV-RT as lower-cost and scalable alternatives to commercial counterparts.

Open-source approaches are also playing a critical role in addressing the unprecedented demand for reagents and infrastructure. During the pandemic, there have been multiple projects developing, open hardware devices (Chagas et al. 2020); open genetic resources such as the European BMRI-ERIC BioBanks and the African Collaborative Initiative to Advance Diagnostics (AFCAD; Peeling et al. 2020) which aims to ensure equitable access to biobank samples for diagnostics developers; as well as biological tools such as the SARS-CoV-2 Genes Collection, the Research in Diagnostics Collection (ReClone) and numerous plasmids and materials that were made available for test development and benchmarking. These initiatives are

often inspired by the free/libre open source software movement and provide freedom to use, study, replicate, and distribute instrumentation, protocols and biomolecular tools with minimal conditions except those required to acknowledge sources, protect patient privacy and comply with laws and regulations.

06.07.02 Open hardware for LAMP

Free and Open-Source Hardware (FOSH) approaches can bring significant cost savings (Pearce, 2016) and allow customization of hardware to meet different experimental and diagnostic needs. For instance, applying FOSH approaches to scientific and medical devices (Baden et al. 2015; Chagas, 2018; http://openhardware.science/; PLOS Open Source Toolkit; Pearce, 2013, Gibb & Abadie, 2014) has led to the development of a range of instruments to undertake each step of an isothermal amplification reaction and some devices now combine steps such as incubation and real-time readout (see section 06.06.02., e.g NinjaLAMP, Miriam, GMO Detective Detector, PocketPCR) while others use simple consumer hardware such as *sous vide* precision cooking devices as low-cost immersion heaters for water baths (Kellner et al. 2020). Other open hardware instruments that have been applied to COVID testing and could scale up throughput of LAMP assays include automation tools such as the OpenTrons OT2 liquid handling robots which have been deployed to accelerate RT-qPCR based testing in Jersey and Pakistan (Walker et al. 2020). Other developments include small devices for specific tasks such as low cost centrifuges used to separate reaction inhibitors from inactivated samples before LAMP (Li et al. 2020), salad spinners to spin down 96/384 well plates (Morán & Galindo, 2011; Motohashi, 2020), and a 3D printed vortex mixer (Patel et al. 2018).

06.07.03 Open reagents and biomolecular tools

Open source approaches have also spanned to free-to-use molecular biology tools, such as plasmid DNA expressing enzymes for RT-LAMP and SARS CoV-2 viral genes distributed by Addgene and Free Genes under open material transfer agreements (Kahl et al. 2018) which enables their use by companies in addition to academics. Other reagents have been distributed freely for non-commercial use (Maranhao 2020b, Alekseenko et al. 2021, Sherril-Mix et al. 2020; Bhadra 2020). ReClone, a reagents collaboration network, has also emerged to provide access to protocols and off-patent reagents, with the aim of mitigating shortages in the short term and building preparedness in the long-term. This network aims to establish an infrastructure to manufacture critical reagents, such as reverse transcriptases (e.g. MMLV, HIV) and Bst LF enzymes used in RT-LAMP. Community biolabs have contributed designs for peripheral hardware such as a simple spectrophotometer and bioreactor developed in the Swiss open public lab, Hackuarium.

Developing and deploying diagnostic tests requires more than reagents, it also requires controls and standards, which many researchers have shared both as data (e.g. DNA sequences) and materials. The Coronavirus Standards Working Group (CSWG) at Stanford University is an

open-membership consortium developing validation panels for diagnostics testing and compiling lists of control materials through public meetings, chat channels and collaboration, similar to other work in genomics standards for laboratory settings (Mason CE, et al, 2017) as well as remote (Nangle S et al., 2020) and harsh environments (Tighe S *et al*., 2017).

The provision of materials by initiatives such as those described above has frequently been coupled to openly published protocols for their use, for example via protocols.io (Teytelman et al. 2016) as mentioned already above, which in comparison to journal article methods sections, offers a much more detailed insight into experimental know-how and the ability to comment, discuss and modify protocols while retaining a version history. Although analysis of these platforms and their use during COVID-19 is only just beginning, this ability to rapidly publish, adapt and republish protocols and materials offers great potential for accelerating innovation at times of crisis where research is taking place under urgent time pressure.

National/International	Bioware/Protocols	Hardware/Software
Global LAMP R&D Consortium (gLAMP)	Various LAMP protocols and methods	https://groups.google.com/g/glamp
Access to COVID Tools Accelerator	Saliva Direct	https://channels.plos.org/open-source-toolkit
UN Technology Access Partnership	https://www.rtlamp.org	http://openhardware.science/
WHO COVID-19 Technology Access Pool	SARS-CoV-2 Genes Collection	GMO Detective Detector
https://wellcome.org/coronavirus-covid-19/open-data	ADDGENE	https://channels.plos.org/open-source-toolkit
Open COVID Pledge	FreeGenes	OpenTrons OT2
Coronavirus Method Development Community	ReClone Research in Diagnostics Collection	Pocket PCR
Coronavirus Standards Working Group	BMRI-ERIC BioBanks	Bomb.bio
Just One Giant Lab JOGL		Open Source Medical Supplies

African Collaborative Initiative to Advance Diagnostics		Helpful Engineering

Table 4. Open Science and Collaborative organizations*. The columns heading are intended only to be an approximate reflection of the focus of the organisation.*

Regulatory Landscape

Regulatory and statutory considerations shape the landscape of clinical diagnostic research, development and deployment. For those seeking to progress their test into clinical use -- from bench to bedside -- we provide this overview of the Regulatory context partly as a case-study for those new to Regulatory Affairs. We highlight the changes arising from the forthcoming EU In Vitro Diagnostic Regulation (IVDR) in May 2022 (Section 07.02.03). As this implies, Regulations are coordinated geographically. We therefore consider first the USA and then the EU, mindful that we leave undiscussed the Regulations that impact the majority of the world's population (e.g. Asia, ASEAN Medical Device Directive).

07.01 Regulatory Perspective in the USA

In the USA, achieving approval from the FDA for a COVID diagnostic test has been a moving target, with significant revisions over the past year following the first major guidance in May 2020 (FDA produced templates for EUA submissions, Table ST4). With so many new laboratory tests being developed, Emergency Use Authorizations (EUAs) were enabled to accelerate the the conventional path to medical device approval given the immediacy and severity of the emerging COVID pandemic (Hasell et al. 2020, https://www.worldometers.info/coronavirus/). To expedite the approval of the growing number of EUA applications, the FDA sought to standardize and clarify a streamlined process with a series of templates being provided over a 6 month period.

Currently, there are 4 primary regulatory pathways for COVID testing in the USA, involving various degrees of oversight:

1. FDA Emergency Use Authorization (EUA);
2. CLIA Laboratory Developed Test (LDT);
3. Testing as a part of a research study conducted under Institutional Review Board (IRB) approval for human subjects research, and
4. Surveillance testing where the test results are **not** returned as if they were from a "CLIA lab" but rather patients with a positive result are referred **to** a CLIA testing facility.

07.01.01 FDA Emergency Use Authorizations (EUA)

EUAs granted by the FDA comprise the (time-bound) regulatory approval for the vast majority of tests for COVID-19. EUAs are issued under a more streamlined process compared to conventional approvals of In Vitro Diagnostic (IVD) tests. For example, tests approved with an EUA do not require GMP/ISO grade reagents (RUO reagents are acceptable) and the size of the clinical validation studies is typically considerably smaller (e.g. 30 positives and 30 negatives for SARS-CoV-2). Individual EUAs for molecular tests for SARS-CoV-2 are classified either as IVDs or LDTs. Whereas LDTs are limited to use in the CLIA laboratory that developed them, IVDs can be sold and used by other laboratories.

At the time of writing, ten LAMP IVD tests have been granted approval under the EUA scheme (Supplementary Table ST1). The first (Atila Biosystems) was a direct swab-based LAMP test, soon followed by a CRISPR-based assay from Sherlock Biosciences, in both cases with fluorescence being monitored using a qPCR or plate-reader. Color Genomics was the first EUA-approved LAMP test to use a 96-well colorimetric readout combined with commercial lysis/inactivation and bead purification reagents. Finally, Pro-Lab Diagnostics nasal swab test with extraction-based or extraction-free ('direct') protocols. When paired with Optigene's GenieHT fluorescence reader the test includes a melting curve analysis of the amplification products, requiring the melting temperature to fall within a specific narrow range (83-85^{o}C). Pro-Lab claims an low limit of detection (LoD) of 125 genome equivalents (GE) per flocked swab (using gamma irradiated cell lysate from BEI Resources).

The LoD has been a key driving metric for SARS-CoV-2 tests and yet is the source of much confusion and not a little controversy. Viral loads (Wofel et al. 2020) typically rise quickly and spike over a day or two and decay more slowly thereafter (Larremore et al. 2020). The infectiousness threshold for replication-competent virus is ~1000 copies/µL (Newman et al. 2020). This corresponds to ~2000-5000 copies/reaction for most RT-LAMP tests, far above the analytical LoD of the reference RT-PCR test using purified RNA (single digit copies/µL). Nevertheless, less sensitive tests can detect the vast majority of those COVID infections that are capable of spreading the virus, especially if used frequently and with ~24 hour turnaround times. The question of what constitutes fit-for-purpose analytical sensitivity for clinical practice remains open (see also Section 8).

To add further confusion, in September 2020, the FDA released the first round of results of a comparison of LoDs for molecular diagnostic tests that had received EUA approval in May 2020. Though dominated by PCR rather than LAMP tests, the LOD varied by four orders of magnitude, with the LODs of many flagship tests being 20-50 fold higher than the LODs being self-reported in their EUA submission documents (see Supplementary Table ST2, Figure S3, Ref: (FDA SARS-CoV-2 Reference Panel Comparative Data - compiled by M. McFarlane SARS-CoV-2 Reference Panel Comparative Data). Three rounds of reference panel LoD results have been released, yet the authors are not aware of any explanation for the large discrepancy in LoD, but this range also matches what is observed from the EUA submissions themselves (MacKay et al. 2020). Currently the reference panel materials (comprising one concentration of heat-inactivated virus and four blinded controls) are only available to EUA holders; wider access to these materials for those making EUA submissions would greatly improve objectivity and might accelerate the validation process.

07.01.02 CLIA and Lab Developed Tests (LDTs)

According to the CDC, "The Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations include federal standards applicable to all U.S. facilities or sites that test human

specimens for health assessment or to diagnose, prevent, or treat disease". The FDA's relationship to regulating LDTs, both for non-emergency declaration tests and EUAs, is complex and evolving. Under normal circumstances, CLIA approved labs can develop LDTs (a.k.a "home brew" tests) that are limited to use in their specific laboratory and require more limited analytical validation data sets. Once the COVID-19 Emergency Declaration by HHS went into effect, CLIA labs were required to submit EUA LDTs. Liability protection (under the PREP Act) and reimbursement (under the FFCRA) both require an EUA. [In August 2020, HHS ruled that the FDA did not have regulatory authority to require pre-market authorization for LDTs under the scheme although this was subsequently reversed on November 16, 2020]. While many labs purchased commercial tests under an existing EUA, others developed and validated their own tests, being allowed to provide the FDA with their validation data in a defined timeframe as part of an EUA request. With a shift in priorities (2nd December 2020) to tests that increase accessibility (e.g. point of care tests) and capacity (high volume tests), it appears that no further LDT EUAs have since been granted authorization.

07.01.03 State Authorization

The FDA is providing flexibility to states who want to authorize labs certified to conduct high-complexity tests in that state to develop and perform coronavirus testing. Under this policy, the state or territory takes responsibility for the safety and accuracy of COVID-19 testing by laboratories in its state/territory, and the lab does not submit an EUA request to the FDA.

07.01.04 Institutional Review Boards (IRBs)

IRBs are formally designated groups that review and monitor clinical research involving human subjects. The purpose of the IRB review is to assure, both in advance and by periodic review, that appropriate steps are taken to protect the rights and welfare of humans participating as subjects in the research. To accomplish this purpose, IRBs use a group process to review research protocols and related materials (e.g., informed consent documents) to ensure protection of the rights and welfare of human subjects of research.

When any researcher is planning to gather personal data and samples from research subjects to validate their lab test (e.g., against a reference like RT-PCR), they must submit their planned study protocol and consent forms to an IRB at their own institution (if they are part of a university) or via a commercial IRB (http://www.circare.org/info/commercialirb.htm). Rules governing the function of an IRB reside within two government agencies - the Office for Human Research Protections (OHRP) and the Food and Drug Administration (FDA) - both under the United States Department of Health and Human Services (HHS).

07.01.05 Surveillance and Asymptomatic Testing

While the regulated pathways of COVID-19 testing have understandably received most attention, a separate pathway has opened up that is not subject to the same regulatory controls.

"Surveillance" testing is primarily used to gain information at a population rather than individual level. It may involve random sampling of a specific population, for example to monitor disease prevalence and determine the population-level effect of community interventions such as social distancing. Prompted by a case involving the Gates Foundation in July/August 2020, the FDA confirmed that if the result of surveillance testing is purely to suggest to an individual that they go for a confirmatory test in a CLIA-certified laboratory, then the surveillance testing itself is not regulated by the CMS or the FDA. In contrast, returning results from tests on asymptomatic people using a CLIA-certified laboratory is regulated by the FDA. While commonly associated with screening of university staff and students, since surveillance testing is not formally regulated in the U.S., it is difficult to estimate the extent of testing performed under this pathway. A subset of the authors are actively engaging in a separate workgroup to explore the potential of expanding this type of RT-LAMP 'surveillance' testing not only in the US but elsewhere.

As the rollout of vaccines continues, U.S COVID-19 testing is projected to remain elevated at least for much of 2021, and with new variants appearing, likely for years beyond. While the role that LAMP tests will play in meeting that demand remains to be seen, continued progress on both the regulatory and commercial fronts will certainly help realize the immense potential of this important and flexible molecular detection technology.

07.02 Regulatory Perspective in the European Union

07.02.01 Current In Vitro Diagnostic (IVD) Medical Device Directive (MDD)

Tests for SARS-CoV-2 are classified as *in-vitro* diagnostic medical devices (IVDs) and must be CE-marked in accordance with the In Vitro Diagnostic Medical Devices Directive IVDD; 98/79/EC before being placed on the market in Europe. CE marking is an administrative marking that indicates conformity with health, safety, and environmental protection standards for products sold within the European Economic Area (EEA) and that it may be legally sold within the EEA, but it is not a quality indicator by itself.

There is no central approval system for *in-vitro* diagnostic medical devices in the EU. For SARS-CoV-2 diagnostics designed for professional use (General IVDs), manufacturers self-certify that their test meets the requirements of the IVD Directive. Manufacturers are required to provide a certificate of conformity and a technical file including the intended use, a risk assessment and the route by which they claim to be in conformity with the Medical Device Directive. The file may also contain a Clinical Evidence Report with sensitivity and specificity claims *etc*. This pathway can be complex and Byzantine given the different regulatory standards and classes of testing devices involved.

The IVD Directive specifies that devices must be designed and manufactured in such a way that they are *suitable for the intended purpose* specified by the manufacturer, taking account of the

generally acknowledged state of the art. They must achieve the relevant performance, in particular in terms of analytical sensitivity, diagnostic sensitivity, analytical specificity, diagnostic specificity, accuracy, repeatability, reproducibility, including control of known relevant interference, and limits of detection, stated by the manufacturer. The test must be registered with the competent authority in the country where they are legally based. A competent authority in the context of IVDs is likely to be a given nation's body with authority to ensure that the requirements of the IVDD are fulfilled in that member state. For COVID-19 devices that are designed for use by lay persons (self-tests), the manufacturer must also apply to a third party body called a notified body who will do additional verification and issue a certificate.

07.02.02 In-house or Lab Developed Tests

Somewhat analogous to US CLIA laboratory standards, ISO15189 is a primary standard that medical laboratories in Europe must adhere to in order to perform clinical testing. These certifications may be held concurrently in the US (Schneider, et al. 2017). Usually, clinical testing laboratories purchase *in-vitro* diagnostic medical devices (IVDs) from market suppliers. However, when pandemic-driven demand for certain IVD products outstrips market supplies of those products, such health institute laboratories may work to develop their own ‘in-house’ or lab developed tests’ (LDTs). IVD tests so developed are not considered to be placed on the market or put into service and are thus exempt from the requirements of Directive 98/79/EC.

Included under this ‘in-house’ exemption:

- IVDs that are manufactured ‘in-house’ are excluded from the legislation if they are used to test patient samples from the same institution.
- If IVDs are manufactured in-house and used to test patient samples from outside of the manufacturing institution (i.e. from external medical practices or other health institutes) they are excluded from the legislation.
- If health institutes use these non CE marked tests the health institute itself must validate the test for their own use.

If these ‘in-house’ manufactured IVDs are transferred to another laboratory and that laboratory is outside the health institutions single legal entity they are then within the scope of the IVD directive and therefore must be CE marked. In this scenario the originating health institution is considered the legal manufacturer of the device (HPRA, 2020).

07.02.03 The upcoming *In Vitro* Diagnostic Regulation (IVDR) 2022

The regulatory landscape described above is changing with the full implementation of the new In Vitro Diagnostic Regulation (IVDR) (EU) 2017/746 as of 26 May 2022 (European Union, 2017). Regulations are being noticeably tightened. After this date, most "general category IVDs" will be reclassified and can no longer be self-certified, including those already on the market. This will increase the percentage of IVDs requiring CE marks from 20% currently under the

IVD Directive to 80% under the IVDR (BSI Group, 2016). In addition, all ‘in-house’ IVDs such as lab developed tests will be regulated, including those that are currently exempt from IVDD. There will be few limited exemptions if an equivalent device available on the market cannot already meet clinical needs at the appropriate level of performance but this scenario will apply to only a very small proportion of LDTs.

In the interest of protection of health, the Directive states that a Member State may, in response to a duly justified request, authorize the placing on the market within its territory of individual devices for which the applicable conformity assessment procedures have not yet been carried out (e.g. pending the completion of the device’s evaluation). In adopting such national derogations, the national competent authority of the Member State must carefully consider any risk against the benefit of having the device available for immediate use. The national processes for adopting these derogations vary across Member States (EU commission, 2020). Any SARS-Cov-2 IVD intended to be used in the EU or to test samples originating in the EU, even if the lab performing the test is based in another country, will be required to comply with IVDR and therefore manufacturers and clinical labs should start preparing for the relevant regulatory submissions with all new tests.

Discussion

The unprecedented demand for diagnostics caused by the COVID-19 pandemic has demonstrated the glaring weaknesses of the global molecular diagnostics infrastructure. Using RT-qPCR as the reference method, some nations fared better than others, but testing needs exposed a strained global supply chain. Delays in turnaround time, limited throughput, inability to cost-effectively scale in many settings and restricted geographical and economic accessibility for both hardware and laboratory supplies showcased the operational weaknesses of RT-qPCR, despite its acknowledged value as a reference method.

As established in other fields and recently in the COVID-19 pandemic, LAMP technology is an accurate, rapid, easily deployable alternative to RT-qPCR, spanning infectious disease, agriculture, food safety, environmental monitoring. Clinically, LAMP has been used within low resource settings e.g. for tuberculosis (WHO, TB-LAMP) and Malaria (Eiken, JP, and Meridian Biosciences Inc, USA, Mohon et al. 2019). The COVID-19 pandemic has driven the need for expanded, lower cost, simple testing solutions from the bench to the bedside and from the Global South to all corners of the globe. Despite sensitivity that is comparable to RT-qPCR -- and importantly, sufficient to detect the most clinically important cases -- LAMP is also not without its limitations and challenges, notably propensity for non-template amplification, complex primer design, and potential for laboratory cross-contamination.

08.01 What is Fit-for-Purpose?

Community-wide, school-based, workplace, at-home, primary clinic, and tertiary hospital testing are each unique milieus, each with their own challenges and considerations. Given the variety of design options available (Figures 1, 8), developers must first define what combination of features are most suited to the intended use (e.g. *total* costs, usability, scalability, accessibility & convenience, diagnostic sensitivity & specificity and effective clinical turnaround time (patient to result)). In resource limited settings, this is paramount, being most clearly articulated in the (RE)ASSURED criteria (Topic Box A).

Some choices are relatively obvious. For a portable testing system, ease of use, small footprint and procedural simplicity is paramount (Newman et al. 2020) while less so for a tertiary hospital. In community schools/colleges etc, it is possible that the staff running the tests may be initially only minimally familiar with molecular biology techniques. In such cases, training materials should fully describe all steps of the process; even basic instructions like "mix" should be expanded to "mix by pipetting," and instructional videos can also be extremely helpful to capture nuances and help staff with troubleshooting. Thus, whether the patient (sample) comes to the test or *vice versa* is a key first-order consideration, closely followed by the experience and training of those performing the testing.

High analytical sensitivity is important when *clinically meaningful* levels of virus are at the limit of detection of the assay. However, if the intended purpose is to identify patients that pose the largest public health risk due to much higher viral loads, then absolute analytical sensitivity is less important. The question of what viral load constitutes contagiousness continues to be debated. Several groups (Dudley et al. 2020, Yaren et al. 2021; Mina, Parker, Larremore, 2020; Mina, M. quoted in Mandavilli, 2020 ; Fowler et al. 2020) have argued that transmission is primarily associated with ~100-fold higher viral loads (≳ 100-1000 copies/uL) than the detection limit of many RT-qPCR reference assays (1-10 copies/uL). This would correspond to a 6-7 Ct 'left-shift' relative to the qRT-PCR limit of detection (Ct ~ 40). Recently Coehlo et al. (2021) presented a thorough analysis of the clinical sensitivity of colorimetric RT-LAMP vs. RT-qPCR on 466 patient swab samples. They conclude that their RT-LAMP assay would reliably assign patients with Ct < 30 as positive/yellow, with Ct > 35 as negative/red and those with 30 < Ct < 35 having an intermediate orange result colour (determined by spectrophotometrically). Taking the estimate from Scola et al. (2020) of Ct > 33-34 as being the threshold for contagiousness, the totality of the data suggests that sensitivities in the 10-100 copies/uL range as observed in LAMP are well within the range required to identify clinically (and epidemiologically) relevant patients. In several recent studies, RT-LAMP has generally demonstrated comparable diagnostic performance to RT-qPCR (Kellner 2021,Natoli 2021, Howson et al. 2021 ; Fowler2021a, Fowler et al. 2021b,Schellenberg 2021,Nawattanapaiboon 2021,Egerer 2021,Coehlo 2021), particularly for samples with the lower, and arguably more clinically relevant, Ct values in RT-PCR, and also shown to be close to RNA-seq metrics (Butler et al. 2021).

Arguably testing with an adequately sensitive method (LAMP) at higher frequency (twice weekly or even daily) to catch infection earlier is preferable to a higher sensitivity test that cannot be administered as often and/or which has a longer patient-to-result turnaround time (RT-qPCR). (Pilcher, 2020 ; Cevik et al. 2021). In the USA, such testing with appropriate reporting of results is free from regulation by the FDA and has enabled surveillance programs in various US colleges. Regardless of frequency, shortening the time between test and actionable result is important (Larremore et al. 2020). The ability of LAMP to provide rapid (~30 min), portable, at- or near-point-of-care testing at a reasonable (and reducing) cost argues for its use in such relatively high frequency, community-based, distributed testing programs. More frequent testing *is* better but a higher sensitivity test is not *necessarily* better. Mining existing clinical and RT-qPCR (C_t value) data to help better address the question of what absolute sensitivity is fit-for-clinical- purpose appears an important but outstanding question, as well as the other biomarkers that can be found to stratify high- and low-risk patients (Ng DL, et al., 2021).

In a similar vein, the clinical test is but one aspect of the clinical diagnosis. False positives that undoubtedly occur, can often be mitigated by repeated testing, particularly if quick and comparatively affordable, or by confirmation using a more laborious and expensive RT-qPCR test on the small number of preliminary positives False positive rates should continue to fall as

the use of dUTP/UDG and sequence-specific detection methods becomes more commonplace (see also Howson et al. 2021). False negatives, on the other hand can be mitigated by more frequent testing and/or contact tracing in situations where testing is applied broadly. Some testing regimens include duplicate testing (one-of-two being considered positive, e.g. Dudley et al. 2020., Newman et al. 2021). Fortunately, LAMP has sufficient sensitivity such that false negatives are comparatively rare at the viral loads generally encountered clinically (> 100 copies/uL).

08.02 Geographical and Economic Accessibility

Any discussion of test sensitivity should consider the cost of *not* testing those patients for whom the 'gold standard' is equally as inaccessible, geographically or economically. Given the transmissibility of the virus (and particularly of newer variants), high testing coverage is in everyone's collective best interests. Driving down the cost and clinical turnaround time (patient-to-result) to allow frequent testing becomes of paramount importance. The manufacture of in-house LAMP reagents by several laboratories argues well for this trend (Section 06.07). Nowhere is this more important than in countries for which access to testing even for even symptomatic patients is limited. In Manchester the cost of the test is likely to be a *secondary* consideration; in Manila can often be the *only* consideration. In remote regions, a hospital with RT-qPCR facility can easily be a day or more away. *The clinical sensitivity of a test that is never run is zero*.

08.04 Attributes of a "Good" RT-LAMP test

We initially sought to answer a seemingly simple question: "*For someone starting to develop a LAMP SARS-CoV-2 assay now, what methodology would we recommend?*". Given LAMP's inherent flexibility and applicability to very different use-cases, making recommendations about "the best method" is necessarily fraught with difficulties. With that caveat, we summarize some of the important technical attributes that a 'good' test would be expected to possess, making reference to the decision points outlined in Figure 1.

(1) Collection method: Several methods have been published using anterior nares swabs (as more convenient alternatives to NPS) and now particularly for saliva/drool (Table 1) and gargle (Kellner et al. 2020 ; www.rtlamp.org). These are reasonable starting points for the vast majority of applications, chosen primarily for their procedural simplicity and the ability of personnel with limited training (or the patient themselves) to perform the sampling. The challenge of saliva is its biological complexity, particularly if used 'directly' without RNA extraction and undiluted (e.g with buffer/saline), and the tropism of each virus is unique, with some more abundant in saliva than others.

(2) Virus Inactivation:, Several simple, low-cost, and effective methods exist (Section 3, Table 1), notably heat inactivation in the presence of a reducing agent, a metal ion chelator and/or a

protease. Importantly, these simple methods enable scalable inactivation at near to the point of collection requiring only limited infrastructure (e.g a heating block) to reduce the required biosafety level for subsequent processing.

(3) RNA Extraction*:* It is here that the developer arguably makes their most important design decision, impacting as it does many upstream (sample selection) and downstream (amplification/detection) steps. The intended use dictates whether to include a dedicated RNA extraction step. Even if so, most LAMP methods do not now use the classical 'RNA extraction kits' used for RT-qPCR testing, partly due to the greater tolerance of LAMP/Bst polymerase to amplification inhibitors and partly to the low-cost, simplified processes of LAMP methods. In resource-limited environments, the time, cost and equipment savings of omitting this step far outweigh the loss in sensitivity (Section 03.04) whereas if resources are available, the increased sensitivity can be useful for e.g. pooling testing schema. Both magnetic and glass milk extractions are viable and increasingly cost-effective (Section 03.03).

(4) Detection chemistry*:* Colorimetric readouts (ΔpH or ΔMg^{2+}_{free}) remain mainstays of LAMP testing primarily due to their simplicity. However, they suffer from sample matrix interferences (phenol red) and/or from difficulties of naked-eye color discrimination (HNB). With the increasing advent of low-cost, more portable fluorescence readers the detection options have increased correspondingly. Of the six main fluorescence-based methods, five are 'sequence-specific' (Table 5) and thus offer the promise of increased matrix robustness, of multiplexing (multiple SARS genes, multiple pathogens and/or an internal process control) and are likely mitigate the very real concern of non-specific amplification (Moehling et al. 2021). These fluorescence approaches provide a 'second stage' of specificity in addition to that from the amplification primers alone (Sherill-Mix et al. (2020)., Bektas et al. (2021)., Bhadra et al. (2020)., Zhang et al.., (2020c). While this increases assay design complexity, we see this category of techniques as the future norm for multiplexed fluorescence-based LAMP detection just as Taqman is for qPCR.

(5) Hardware/Software for Quantification*:* Both colorimetric and fluorigenic LAMP tests can now be objectively quantified with a range of smartphone/tablet applications (Section 06.04) and a slew of mid-priced dedicated incubator/fluorescence readers, some with melt curve capabilities. From the simplest water-bath to low cost plate- and tube-based heaters (many being open source) to more advanced microfluidic platforms, there is no shortage of hardware options for the relatively simple process of maintaining a LAMP reaction at one or two elevated temperatures (Section 06.03). With a reasonably small budget, one can now afford the infrastructure to perform sequence-specific, multicolor multiplexed fluorescence based (kinetic) LAMP assays with one or more targets and an internal extraction control. Arguably if LAMP is to attain the widespread clinical penetration enjoyed by multiplexed qPCR this type of low-cost experimental set-up will need to become the norm.

Test principle	**ΔpH**	**$\Delta[Mg^{2+}]_{free}$**	**dsDNA Intercalation**	**Sequence-specific fluorescence**	**DNA sequencing**
Review Section	05.01	05.01	05.01	05.02	05.03
Example reference	Zhang[b]	Kellner	Diego	Bektas/Zhang[c]/Bhadra/ Yaren/Sherill-Mix	James
Method/dye	Phenol red LSV/LSM	HNB eBT	SYTO9 Eva/SYBR Green/ LCV	QUASR/DARQ/OSD /DP/MolBeac	Sequencing LAMP
Specimen impact					
1. **R**eal-time connectivity					
2. **A**ffordable					
3. **S**ensitive					
4. **S**pecific					
5. **U**ser-friendly					
6. **R**apid/**R**obust					
7. **E**quipment free					
8. **D**eliverable to end-users					

Table 5: Qualitative comparison of methods: *Each method is broadly rated against the REASSURED criteria (see Topic Box A) supplemented with an assessment of the impact of interferences introduced via the Sample specimen. We assume that the use of Smartphones to capture data and relay results will become increasingly ubiquitous. For clarity, the qualitative coloring scheme is only intended to be indicative, primarily to provide the Reader with a high-level appreciation of the choices and trade-offs that the developer makes when selecting an assay format. Exceptions to these broad categorizations are expected to be legion. LSV/M: Lampshade Violet/Magenta; HNB: hydroxynaphthol blue; eBT: eriochrome Black T; EvaGreen/SYBR Green/SYTO9: Cyanine-dye based (green) fluorescent dyes; LCV: Leucocrystal violet; QUASR: Quenching of Unincorporated Amplicon Signal Reporters; DARQ: Quenching of Unincorporated Amplicon Signal Reporters OSD: One-step Strand Displacement; DP Displaceable probes ; MolBeac: Molecular Beacons. Combination methods of LAMP + CRISPR-Cas (e.g. Broughton et al. 2020) or of RPA + LAMP (El Tholoth et al. 2020) are omitted.*

08.05 Future Technical Directions

As vaccinations are becoming more available and accessible, novel variants with increased transmissibility and infectivity remind us that diagnostics remain an integral component to our response to COVID-19 (Alpert et al., 2021) and integration with changes in clinical care (Afshinnekoo E., *et al*., 2021). The last year has seen the maturation of LAMP-based diagnostics from the bench to the bedside and from the Global South to all corners of the globe. Isothermal methods have a number of inherent logistical advantages over qPCR and yet LAMP has several remaining challenges to address for more widespread use in a clinical setting. Even relatively low rates of false positives caused by mis-priming (or cross-contamination) in LAMP assays represents an Achilles Heel for diseases with relatively low (post-pandemic) prevalence (Moehling et al. 2021). It is for this reason that we highlight the variety of sequence-specific fluorescence-based methods (Section 05.02) as a likely future trajectory for clinical LAMP testing.

The intrinsic biochemical sensitivity of LAMP is directly comparable to that of qPCR (single digit copies per reaction). However, many LAMP developers have consciously addressed a different, near point of care, use case with minimal sample processing. This contrasts with the highly resource and automation-intensive methods required in central RT-qPCR testing facilities that perform RNA extraction as a matter of clinical normality. Given this fundamental difference in objective, the question regarding what represents a *fit-for-that-purpose* LAMP test sensitivity remains. Focusing excessively on sensitivity as but one of the eight REASSURED dimensions appears unnecessarily self-limiting. The effective sensitivity of a qPCR test that is never deployed because it is geographically or economically inaccessible is far below that of an accessible but lower sensitivity LAMP test; it is zero.

High quality rapid antigen tests clearly have clinical utility. If advances in LAMP technology, reagents and supply chains made over the past 12 months continue, we can foresee a time when LAMP molecular tests can compete with all but the cheapest rapid antigen tests. Since LAMP tests provide the starting material for direct sequencing of positives, it provides a strong rationale for their wider adoption, particularly as the price differential with rapid Ag tests continues to diminish. This leaves the door open for wider-scale identification of new COVID-19 variants, emerging pathogens, and a wide variety of otherwise undetectable public health threats. The ability to sequence directly from LAMP reactions (LAMP-Seq/LAMPore) also distinguishes it favorably from qPCR (Section 05.03).

08.06 Conclusion

If we have learned anything in the past year, it is that *existing diagnostic systems and implementations globally have failed the enormous challenge posed by SARS-CoV-2 and that a massive increase in rapid testing methods are needed to mitigate spread of viruses***.** Whether testing more symptomatic patients in resource limited settings or more asymptomatic individuals

in countries where testing is more widely available, there needs to be a shift in thinking to include regular repeated testing of large swaths of the population.

The flexibility to create multiple versions of LAMP suited to different settings is key. Such flexibility does more than create design and supply chain choices, it fundamentally changes the landscape of what is possible for infectious disease. The potential to expand testing opens up public health responses and policies that have until now seemed unrealizable. Parallels to the transition of computing from one of large, centralized mainframe systems to include, and then become dominated by, personal computing are quite clear. New technologies provided individuals and small groups with access to computational power previously centralized in large companies. As a trend, we embrace a more distributed future for diagnostics where local entities can perform work that was previously reserved for large, highly resourced labs.

Commensurate with the focus of the Journal, we have sought to highlight some of the more relevant and innovative methodological advances to enable such a sea-change. While much remains to be done, LAMP methods have greatly matured in the past year, with increasing acceptance that it can now take its place squarely in the cast of available clinical technologies in clinical labs. With a broad and vibrant Open Science movement enabling lower cost innovations in bioware, software and hardware, the accessibility of diagnostics for a larger proportion of the globe is an increasingly realistic possibility.

If the future of testing is indeed distributed and closer to the point of patient care, the inherent advantages of LAMP mean it can play a significant role just as PC’s (and later laptops and then cell phones) did for information and communication. Whatever the ultimate trajectory of diagnostic testing, lessons learned need to continue to be translated into robust and reliable clinical testing platforms. While we will eventually curtail the spread of SARS-CoV-2, the next major infectious agent is surely on the horizon somewhere on our increasingly small and interconnected globe.

Topic Box D: Review Highlights and Takeaways

This Box highlights some important considerations for building LAMP tests based on the scale of testing, the intended use, location/setting, budget and level of infrastructure available.

a. **The right test for the right application: "*We have a LAMP for that*".** Given the variety of design options available, the testing problem dictates the most appropriate testing configuration. Unlike the qPCR platform, LAMP testing schema can vary widely in total costs, usability, scalability, accessibility & convenience, diagnostic sensitivity & specificity and effective clinical turnaround time (patient to result).
b. **How will the test result be used?** For clinical diagnosis of the individual or epidemiological surveillance of a population? Testing in singlicate or pooled format? These very different uses dictate consciously-made decisions about tradeoffs between sensitivity, specificity, speed of result and total cost of testing.
c. **How does the hidden cost of *not* testing affect utility?** If your test is geographically or economically inaccessible to the many, it is of far less aggregate value than a "high quality" test that is accessible only to the few. The logistical advantages of LAMP over qPCR have a real clinical value: *the sensitivity of a test that is never run is zero.*
d. **Location, location, location!**: The inexorable drive to distributed testing requires designs that are "As simple as possible… but no simpler". With hardware getting smaller, more portable and cheaper and readouts becoming both simple and specific, impediments to testing based in geographical inaccessibility will continue to diminish.
e. **Frequency and time-to-results matter**. Once demand for symptomatic testing is met, twice weekly testing in congregate living settings should be an achievable objective of many testing regimes. The viral load rises quickly in a newly infected person so frequent testing is key to containment. If possible, take the test to the patient, not the other way around, particularly for use in remote/rural locations.
f. **Being positive and being contagious may not be the same thing**. Basing clinical and policy decisions on the analytical sensitivity of qPCR might not be the most clinically relevant measure of test utility. If the purpose is to identify individuals with the highest probability of transmission then a LAMP test with sufficient test sensitivity but higher population accessibility likely has the more preferable overall profile.
g. **Think beyond a single test**. The diagnostic test as evaluated in the literature is only a partial reflection of clinical practice. Repeat or duplicate testing improves clinical diagnosis, either with the same test (LAMP → LAMP) or with an orthogonal test (e.g

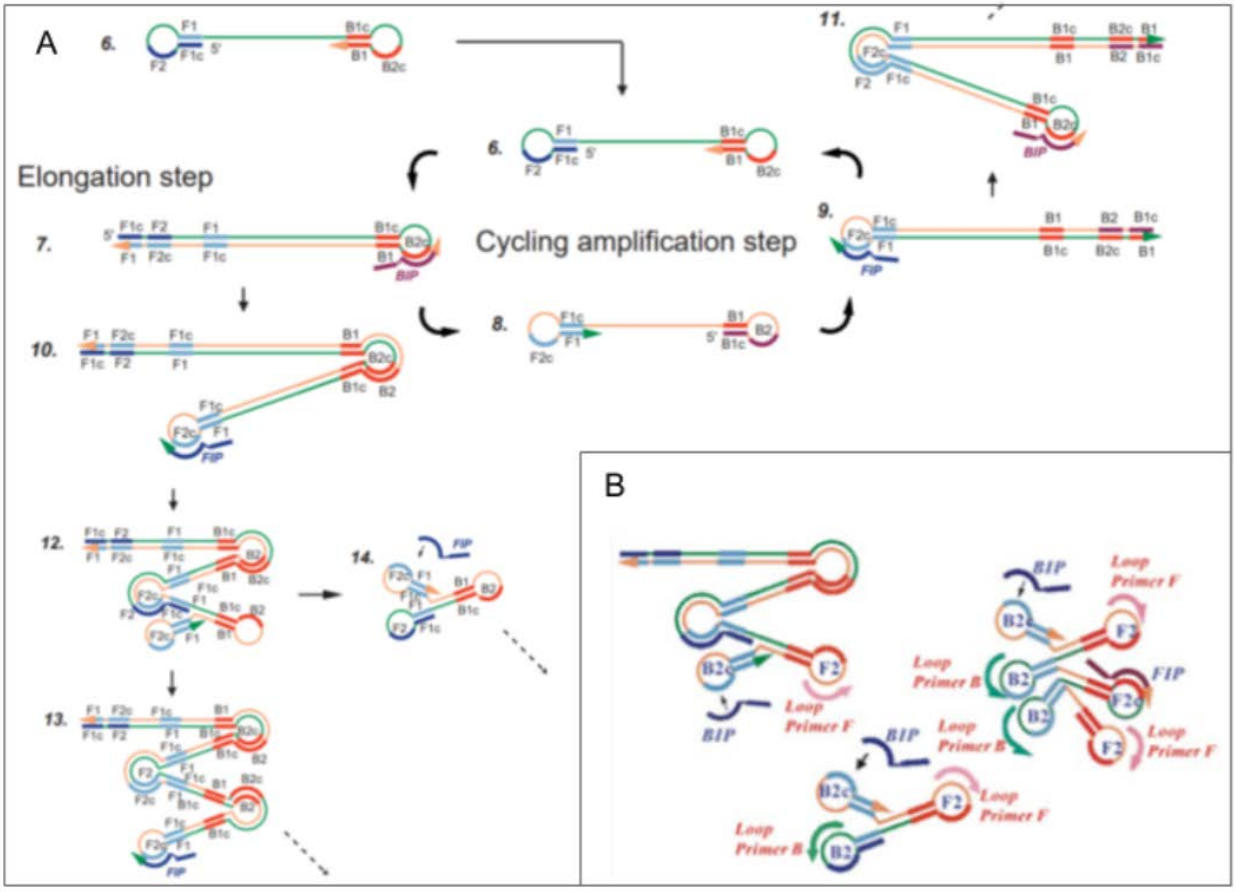
LAMP → PCR). If the test is local/distributed, relatively fast, and relatively cheap, confirmation/repeats of positives is a viable triaging strategy.

h. **Think beyond testing personnel in the lab:** As testing becomes increasingly distributed (either by choice or out of necessity), the test is likely to be run by healthcare workers with minimal formal training in molecular biology techniques. Take nothing for granted, ensure user acceptance testing occurs in the intended settings and consider the increasing accessibility of video as a communication and training tool.

i. **Attributes of a 'good\ SARS-CoV2 RT-LAMP test:**
    a. *Collection method:* Saliva/gargle or OP/AN swabs are validated biospecimens and highly preferable to NP swabs due to simplicity and impact on the patient.
    b. *Viral inactivation;* There are several methods for viral inactivation based on heating, typically in TCEP/EDTA/ProteinaseK buffers to stabilize viral RNA.
    c. *RNA Extraction/purification:* In resource limited environments, the savings in cost/equipment/training far outweigh the loss in sensitivity associated with omitting the extraction step. With greater resources and if increased sensitivity adds value (e.g. for pooling samples), simplified RNA extraction methods based on magnetic and glass milk/silica beads are available.
    d. *Control RNA:* RNA, heat- and gamma radiation-inactivated viral controls from BEI Resources are a good starting point for preclinical method development.
    e. *Target and control genes:* Most LAMP assays use one or more Nucleocapsid (N) primer sets, alone or augmented with Envelope (E), RdRp and sometimes Spike (S) sets. RnaseP and beta-actin are common positive extraction controls.
    f. *Reaction monitoring:* If using a pH-based method, neutralize the input samples to avoid false positives; if using a colorimetric readout, use Smartphone apps to quantify color changes; if using fluorescence, newer sequence-specific methods are increasingly preferred over dye intercalation to reduce false positives.
    g. *Hardware/Software:* Every day seemingly brings new hardware and software, both from commercial suppliers of lower cost alternatives to qPCR readers and labs taking advantage of the Free and Open Source Hardware movement.

Supplementary Information

Figure S1



Supplementary Figure S1. LAMP Cycling amplification at a constant temperature. A*) The dumbbell structures created in the initial reaction (6 and 8, see Figure 1) are then elongated through self-priming creating stem-loop structures (7 and 9). Binding/annealing of the FIP/BIP to the single-stranded loop (F2c/B2c) primes strand displacement DNA synthesis releasing the previously synthesized strand. This released single strand forms a new stem-loop structure folding in on itself because of complementarity of F1/B1 and F1c/B1c which is then elongated releasing the previously extended FIP/BIP which now again creates a new dumbbell structure (6 and 8 cycling step) as well as a longer stem loop (10 and 11) which can then be amplified again, creating various sized structures consisting of alternately inverted repeats of the target sequence on the same strand (12-14).* ***B)*** *The addition of loop primers accelerates LAMP reactions. The region between F1 and F2 (or B1c and B2c) is hybridized by the loop primer and allows more rapid amplification. For example, in the original method, only two of the six single stranded loops would be used to initiate amplification, with the addition of loop primers all six are utilized.*

S2: Primer design considerations

Some considerations when designing primers (based on Ref. S1 and Ref S2 below)

Melting Temperature (T_m)

Generally, the T_m should match for the following pairs: F2/B2, F1c/B1c, and F3/B3. The T_m for each region is designed to be approximately 65°C (64 - 66°C) for F1c and B1c, approximately 60°C (59 - 61°C) for F2, B2, F3, and B3, and approximately 65°C (64 - 66°C) for the LB and LF. However, in AT-rich sequences (GC content less than 45%), the T_m for F3, F2, B3, and B2 should be lowered to approximately 57°C, while the T_m for F1c and B1c should be lowered to 52°C.

Lastly, the T_m is ideally calculated under standardized experimental conditions, such as 50mM Na^+, 4mM Mg^{2+}, 10^{-7} M primer concentration, etc.

End Stability

The 3' ends of F2/B2, F3/B3, and LF/LB and the 5' end of F1c/B1c are designed so that the free energy is –4 kcal/ mol or less.

GC Content and Predicted Secondary Structure

Primers are designed to have GC contents ranging from 40% to 65%, but GC contents between 50% to 60% tend to produce relatively good primers. It is also important to avoid primer dimers in predicted secondary structures (e.g. 3' ends with 2 or more self-hybridizing bases should be avoided, or 3' ends should not be complementary).

Design Considerations for QUASR probes

1. Melting Temp (T_m)
- The T_m for each region is designed to be approx. 65°C (64 - 66°C) for F1c and B1c, approx. 60°C (59 - 61°C) for F2, B2, F3, and B3, and approx. 65°C (64 - 66°C) for the loop primers
- AT-rich sequences: F3,2 + B3,2: 57C; F1c + B1c: 52C
- Difference in T_m should be $\leq 5^oC$ between F2 and F1c and between B2 and B1c
- T_m should match for F2+B2, F1c+B1c, and F3+B3
- Melting temperatures should be estimated under standardized conditions i.e. 10^{-7} M primer, 50mM Na^+, 4 mM Mg^{2+}.

2. End Stability
- The 3' ends of F2/B2, F3/B3, and LF/LB and the 5' end of F1c/B1c are designed so that the free energy is –4 kcal/ mol or less.

3. GC Content and Predicted Secondary structure
- GC content should be between 40-60% (preferably 50-60%)
- Predicted secondary structure or primer dimers should be avoided (e.g. 3' ends with 2 or more self-hybridizing bases should be avoided)

Design considerations specifically for QUASR probes (based on Ref. S3 and Ref. S4 below)

- Quencher probe is typically 7-13 bases in length and is complementary to the 5' end of the fluorescently labeled probe
- T_m of the quencher probe to the fluorescently labelled primer should be <55ºC (well below 63-65ºC reaction temperature)
- Quenching probes should be added in molar excess to ensure fully quenching of the labelled primer in the absence of incorporation.
- Fluorescently labelled FIP and BIP generally provide a brighter signal than labelled LB or LF

References

NEB: http://primerexplorer.jp/e/v5_manual/pdf/PrimerExplorerV5_Manual_1.pdf
Lucigen: https://www.lucigen.com/docs/slide-decks/Lucigen-LAMP-Loop-Mediated-Isothermal-Amplification-Webinar-Slidedeck.pdf
Ball et al. (2016) : *Anal. Chem.* 2016, 88, 7, 3562–3568., https://doi.org/10.1021/acs.analchem.5b04054
Meagher, R et al. (2018) *Analyst*, 2018,143, 1924-1933., https://doi.org/10.1039/C7AN01897E

Supplementary Table ST1

EUA Date	Test	Diagnostic	Target	IC	Detection	Extraction	Amplification	LOD spike	LOD GE/ 3ml swab
Nov-17	Lucira (At Home POC)	Lucira COVID-19 All-In-One Test Kit	N, N	Lysis Ctrl + IC	Colorimetric	~Direct	Lucira device	Inactivated virus	2,700*
Oct-05	Seasun (2 duplex)	AQ-TOP COVID-19 Rapid Detection Kit PLUS	orf1ab, N	Human RNaseP	PNA Probe	Manual (Qiagen_60704 or Seasun_SS-1300) or Automated (Panagene_PNAK-1001 on PanaMax48)	BioRad_CFX96 or ABI7500	NCCP_43326 genomic RNA	3,000
Sep-01	Detectachem	MobileDetect Bio BCC19 (MD-Bio BCC19) Test Kit	N, E	only external controls	Colorimetric	Direct (1µl of transport media into LAMP)	heat block or qPCR (MD-Bio heater or BioRad_T100 or AB_Veriti or …)	Twist_MT007544.1 synthetic gRNA	225,000
Aug-31	Mammoth	SARS-CoV-2 DETECTR Reagent Kit	N	Human RNaseP	CRISPR Probe	Automated (Qiagen_955134 on Qiagen_EZ1AdvancedBenchtop)	ABI7500	SeraCare_AccuPlex_0505-0168 IVT encapsulated RNA	60,000
Aug-13	Pro-Lab	Pro-AmpRT SARS-CoV-2 Test	RdRP	only external controls	Probe	Direct (swab into 0.1ml) or kit (swab into 1ml then Pro-Lab_PLM-2000)	Optigene_GenieHT	BEI_NR-52287 inactivated virus	125*
Jul-09	UCSF/Mammoth	SARS-CoV-2 RNA DETECTR Assay	N	Human RNaseP	CRISPR Probe	automated (Qiagen_955134 on Qiagen_EZ1)	ABI7500	SeraCare_AccuPlex_0505-0126 (lot 10480311) IVT RNA	60,000
May-21	Seasun (1 duplex)	AQ-TOP COVID-19 Rapid Detection Kit	orf1ab	Human RNaseP	PNA Probe	manual (Qiagen_60704)	BioRad_CFX96 or ABI7500	NCCP_43326 gRNA	21,000
May-18/ Aug31	Color	Color Genomics SARS-CoV-2 RT-LAMP Diagnostic Assay	N, E, nsp3	Human RNaseP	Colorimetric	automated (PerkinElmer_CMG-1033 on PerkinElmer_Chemagic360)	plate reader (Biotek_NEO2), Hamilton_Star	ATCC_VR-1986D (gRNA)	2,250
May-06	Sherlock BioSci	Sherlock CRISPR SARS-CoV-2 Kit	orf1ab, N	Human RNaseP	CRISPR Probe	Manual (ThermoFisher_12280050)	heat block or qPCR, Plate reader (BioTek_NEO2)	gRNA	20,250
Apr-10	Atila BioSystems	iAMP COVID-19 Detection Kit	orf1ab, N	Human GAPDH	Probe	Direct (swab into 350µl, 15min RT then 3µl to LAMP)	BioRad_CFX96 or ABI7500 or Roche_LightCycler480II or Atila_PG9600	SeraCare_AccuPlex_0505-0129 pseudovirus (recombinant alphavirus)	3,500*

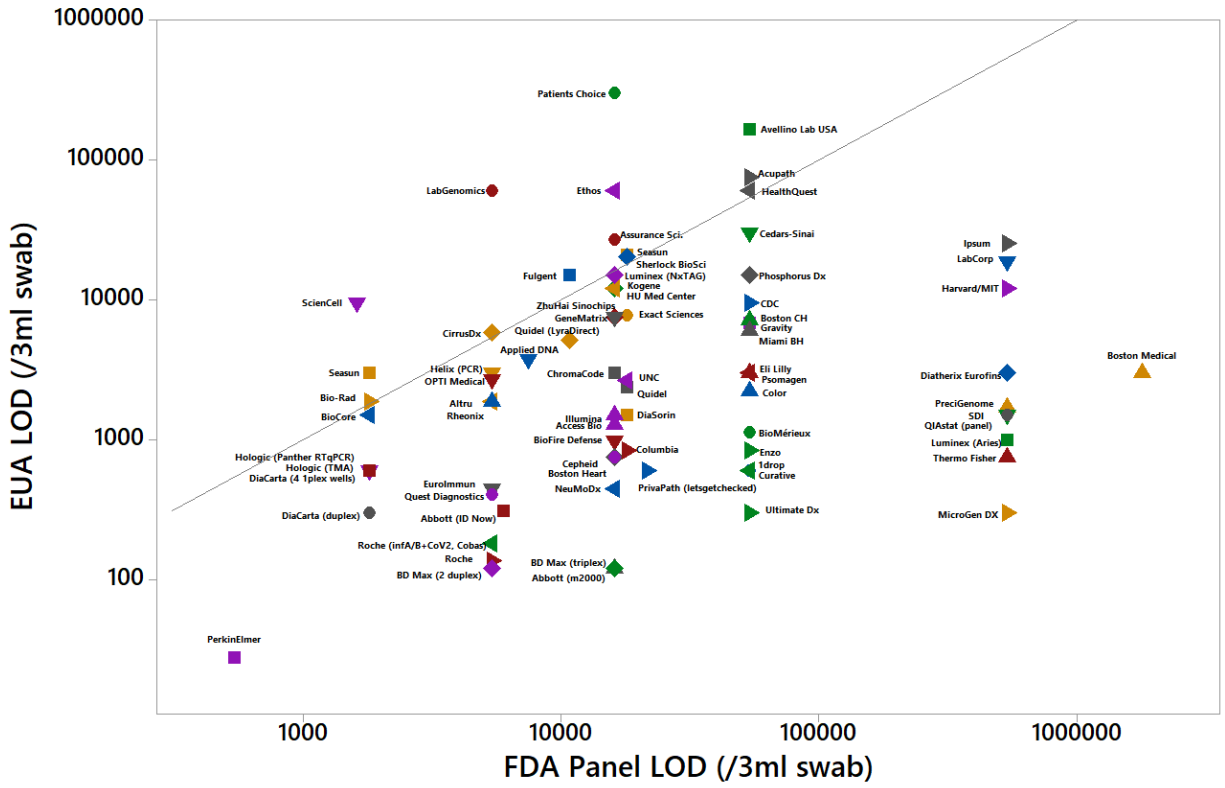
*Lucira, Pro-Lab and Atila tests LOD/swab uses EUA specified volumes which are less than 3ml 1

Supplementary Table ST2

This is the raw data from which this graph is derived.

https://docs.google.com/spreadsheets/d/1Y1H4bPgi-xeD08LaMvvtvQhyj6vchuf1tObI9KTyKcw/edit?usp=sharing

Supplementary Figure S3



Guidance Document	Scope	Last Update
Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised)	This guidance describes a policy for laboratories and commercial manufacturers to help accelerate the use of tests they develop in order to achieve more rapid and widespread testing capacity in the US.	May 11, 2020
Home Specimen Collection Molecular Diagnostic Template	This template provides the FDA recommendations concerning what data and information should be submitted to FDA in support of a pre-EUA/EUA submission for prescription use only home collection devices used by an individual to collect certain clinical	May 29, 2020

	specimen(s) that are then sent to a clinical laboratory for testing with a molecular diagnostic for SARS-CoV-2 that is authorized for use with the home collection kit.	
Molecular Diagnostic Template for Commercial Manufacturers	Provides FDA's recommendations concerning what data and information should be submitted to FDA in support of a pre-EUA/EUA submission for a molecular diagnostic for SARS-CoV-2.	July 28, 2020
Molecular Diagnostic Template for Laboratories	Includes FDA's recommendations for laboratories concerning what data and information they should submit to support an EUA request for a molecular diagnostic for SARS-CoV-2 developed for use in a single CLIA certified high-complexity laboratory.	July 28, 2020
Template for Manufacturers of Molecular and Antigen Diagnostic COVID-19 Tests for Non-Laboratory Use	Provides FDA's recommendations concerning what data and information should be submitted to FDA in support of a pre-EUA/EUA submission for a molecular or antigen diagnostic test for SARS-CoV-2 for use in a non-laboratory setting. Such settings are likely to include a person's home or certain non-traditional sites such as offices, sporting events, airports, schools etc. This template does not apply to home collection kits.	July 29, 2020

Table ST4. *Summary of COVID-19 United States Regulatory Guidance Documents*

Item	Catalog Number	Cost	# tests	$/test
Pipette Tips 200ul case	76322-160	$275	4800	$0.06
100ul Pipette	76335-742	$292	N.A	NA
Lab Biohazard bag	10035-976	$38	N.A	NA
PCR Plates (200ul tubes for lower throughput)	10049-108	$125	2400	$0.05
Colorimetric LAMP	e.g NEB E2019S	$750	96	~$8
65°C heat source	Sous vide/water	~ $40	N.A	N.A
nitrile gloves	89428-750	$228	2000	$0.11
1.5 ml eppendorf Lo-bind	80077-230	$32	250	$0.13

Eppendorf Thermocycler	71003-564	$8,600
Fume Hood	89260-050	$10,300
Mechanical Convection Oven	Thermo Fisher PR305220M	$2,900
Plate Reader	Biotek Neo 2	$34,600
Corning LSE Vortex Mixer	6775	$150
Corning LSE Mini Microfuge	6770	$150

Refrigerated Centrifuge	5942000245	$16,140
BioTek NEO2S	BTNEO2S	$42,100
PX1 PCR Plate Sealer	1814000	$4,180
Xpeel plate seal remover	1150L21	$35,800
BioTek™ MultiFlo FX MultiMode Dispenser	BTMFXP1	$17,400
Hamilton Starlet liquid handling robot (LHR)		$140,000
Chemagic 360 (if performing magnetic-bead based nucleic acid extraction)	2024-0020	$128,000

Bibliography

1. Abraham, J. P., Plourde, B. D., & Cheng, L. (2020). Using heat to kill SARS-CoV-2. *Reviews in medical virology*, *30*(5), e2115. https://doi.org/10.1002/rmv.2115
2. Afshinnekoo E, Bhattacharya C, Burguete-García A, Castro-Nallar E, Deng Y, Desnues C, Dias-Neto E, Elhaik E, Iraola G, Jang S, Łabaj PP,; MetaSUB Consortium. COVID-19 drug practices risk antimicrobial resistance evolution. *Lancet Microbe*. 2021 Apr;2(4):e135-e136.
3. Agrawal, S., Fanton, A., Chandrasekaran, S., Charrez, B., Escajeda, A., Son, S., Mcintosh, R., Bhuiya, A., Le'on Derby, M., Switz, N., Armstrong, M., Harris, A., Prywes, N., Lukarska, M., Biering, S., Smock, D., Mok, A., Knott, G., Dang, Q., Van Dis, E., Dugan, E., Kim, S., Liu, T., , Harris, E., Stanley, S., Lareau, L., Tan, M., Fletcher, D., Doudna, J., Savage, D., & Hsu, P. (2021). Rapid, point-of-care molecular diagnostics with Cas13. *medRxiv*.
4. Aidelberg, G., & Aronoff, R. (n.d.). *Freeze-drying (Lyophilization) of CoronaDetective tubes V.1*. Freeze-drying (Lyophilization) of CoronaDetective tubes. https://www.protocols.io/view/freeze-drying-lyophilization-of-coronadetective-tu-bk44kyyw.html.
5. Alekseenko, A., Barrett, D., Pareja-Sanchez, Y., Howard, R. J., Strandback, E., Ampah-Korsah, H., Rovšnik, U., Zuniga-Veliz, S., Klenov, A., Malloo, J., Ye, S., Liu, X., Reinius, B., Elsässer, S. J., Nyman, T., Sandh, G., Yin, X., & Pelechano, V. (2021). Direct detection of SARS-CoV-2 using non-commercial RT-LAMP reagents on heat-inactivated samples. *Scientific reports*, *11*(1), 1820. https://doi.org/10.1038/s41598-020-80352-8
6. Alekseenko, A., Barrett, D., Pareja-Sanchez, Y., Howard, R. J., Strandback, E., Ampah-Korsah, H., Rovšnik, U., Zuniga-Veliz, S., Klenov, A., Malloo, J., Ye, S., Liu, X., Reinius, B., Elsässer, S. J., Nyman, T., Sandh, G., Yin, X., & Pelechano, V. (2021). Direct detection of SARS-CoV-2 using non-commercial RT-LAMP reagents on heat-inactivated samples. *Scientific reports*, *11*(1), 1820. https://doi.org/10.1038/s41598-020-80352-8
7. Allgöwer, S. M., Hartmann, C. A., Lipinski, C., Mahler, V., Randow, S., Völker, E., & Holzhauser, T. (2020). LAMP-LFD Based on Isothermal Amplification of Multicopy Gene *ORF160b*: Applicability for Highly Sensitive Low-Tech Screening of Allergenic Soybean (*Glycine max*) in Food. *Foods (Basel, Switzerland)*, *9*(12), 1741. https://doi.org/10.3390/foods9121741
8. Alpert T, Brito AF, Lasek-Nesselquist E, Rothman J, Valesano AL, MacKay MJ, Petrone ME, Breban MI, Watkins AE, Vogels CBF, Kalinich CC, Dellicour S, Russell A, Kelly JP, Shudt M, Plitnick J, Schneider E, Fitzsimmons WJ, Khullar G, Metti J, Dudley JT, Nash M, Beaubier N, Wang J, Liu C, Hui P, Muyombwe A, Downing R, Razeq J, Bart SM, Grills A, Morrison SM, Murphy S, Neal C, Laszlo E, Rennert H, Cushing M, Westblade L, Velu P, Craney A, Cong L, Peaper DR, Landry ML, Cook PW, Fauver JR, Mason CE, Lauring AS, St George K, MacCannell DR, Grubaugh ND. Early introductions and transmission of SARS-CoV-2 variant B.1.1.7 in the United States. *Cell*. 2021 Apr 3: S0092-8674(21) 00434-7.
9. Augustine, R., Hasan, A., Das, S., Ahmed, R., Mori, Y., Notomi, T., Kevadiya, B. D., & Thakor, A. S. (2020). Loop-Mediated Isothermal Amplification (LAMP): A Rapid, Sensitive, Specific, and Cost-Effective Point-of-Care Test for Coronaviruses in the Context of COVID-19 Pandemic. *Biology*, *9*(8), 182. https://doi.org/10.3390/biology9080182
10. Ball, C. S., Light, Y. K., Koh, C. Y., Wheeler, S. S., Coffey, L. L., & Meagher, R. J. (2016). Quenching of Unincorporated Amplification Signal Reporters in Reverse-Transcription Loop-Mediated Isothermal Amplification Enabling Bright, Single-Step, Closed-Tube, and Multiplexed Detection of RNA Viruses. *Analytical chemistry*, *88*(7), 3562–3568. https://doi.org/10.1021/acs.analchem.5b04054
11. Basiri, A., Heidari, A., Nadi, M. F., Fallahy, M., Nezamabadi, S. S., Sedighi, M., Saghazadeh, A., & Rezaei, N. (2021). Microfluidic devices for detection of RNA viruses. *Reviews in medical virology*, *31*(1), 1–11. https://doi.org/10.1002/rmv.2154
12. Batéjat, C., Grassin, Q., Manuguerra, J. C., & Leclercq, I. (2021). Heat inactivation of the severe acute respiratory syndrome coronavirus 2. *Journal of biosafety and biosecurity*, *3*(1), 1–3. https://doi.org/10.1016/j.jobb.2020.12.001
13. Becherer, L., Borst, N., Bakheit, M., Frischmann, S., Zengerle, R., & Stetten, F. (2020). Loop-mediated isothermal amplification (LAMP) – review and classification of methods for sequence-specific detection. *Anal. Methods, 12, 717-746.*
14. Bekta\c s, A., Covington, M., Aidelberg, G., Arce, A., Matute, T., N\'u\~nez, I., Walsh, J., Boutboul, D., Lindner, A., Federici, F., & Jayaprakash, A. (2021). Accessible LAMP-Enabled Rapid Test (ALERT) for detecting SARS-CoV-2. *medRxiv*.
15. Ben-Assa, N., Naddaf, R., Gefen, T., Capucha, T., Hajjo, H., Mandelbaum, N., Elbaum, L., Rogov, P., King, D. A., Kaplan, S., Rotem, A., Chowers, M., Szwarcwort-Cohen, M., Paul, M., & Geva-Zatorsky, N. (2020). Direct on-the-

spot detection of SARS-CoV-2 in patients. *Experimental biology and medicine (Maywood, N.J.)*, *245*(14), 1187–1193. https://doi.org/10.1177/1535370220941819

16. Berkenbrock, J. A., Grecco-Machado, R., & Achenbach, S. (2020). Arsenal of microfluidic testing devices may combat COVID-19 pandemic. *MRS bulletin*, *45*(7), 511–514. https://doi.org/10.1557/mrs.2020.181
17. Bhadra, S., Maranhao, A. C., Paik, I., & Ellington, A. D. (2020). One-Enzyme Reverse Transcription qPCR Using Taq DNA Polymerase. *Biochemistry*, *59*(49), 4638–4645. https://doi.org/10.1021/acs.biochem.0c00778
18. Bhadra, S., Riedel, T., Lakhotia, S., Tran, N., & Ellington, A. (2020). High-surety isothermal amplification and detection of SARS-CoV-2, including with crude enzymes. *bioRxiv*.
19. Bokelmann, L., Nickel, O., Maricic, T., Pääbo, S., Meyer, M., Borte, S., & Riesenberg, S. (2021). Point-of-care bulk testing for SARS-CoV-2 by combining hybridization capture with improved colorimetric LAMP. *Nature communications*, *12*(1), 1467. https://doi.org/10.1038/s41467-021-21627-0
20. Broughton, J. P., Deng, X., Yu, G., Fasching, C. L., Servellita, V., Singh, J., Miao, X., Streithorst, J. A., Granados, A., Sotomayor-Gonzalez, A., Zorn, K., Gopez, A., Hsu, E., Gu, W., Miller, S., Pan, C. Y., Guevara, H., Wadford, D. A., Chen, J. S., & Chiu, C. Y. (2020). CRISPR-Cas12-based detection of SARS-CoV-2. *Nature biotechnology*, *38*(7), 870–874. https://doi.org/10.1038/s41587-020-0513-4
21. Brune Z, Kuschner CE, Mootz J, Davidson KW, Pena RCF, Ghanem MH, Fischer A, Gitman M, Teperman L, Mason C, Becker LB. Effectiveness of SARS-CoV-2 Decontamination and Containment in a COVID-19 ICU. Int J Environ Res Public Health. 2021 Mar 3;18(5):2479.
22. Bull, R. A., Adikari, T. N., Ferguson, J. M., Hammond, J. M., Stevanovski, I., Beukers, A. G., Naing, Z., Yeang, M., Verich, A., Gamaarachchi, H., Kim, K. W., Luciani, F., Stelzer-Braid, S., Eden, J. S., Rawlinson, W. D., van Hal, S. J., & Deveson, I. W. (2020). Analytical validity of nanopore sequencing for rapid SARS-CoV-2 genome analysis. *Nature communications*, *11*(1), 6272. https://doi.org/10.1038/s41467-020-20075-6
23. Buonanno, G., Stabile, L., & Morawska, L. (2020). Estimation of airborne viral emission: Quanta emission rate of SARS-CoV-2 for infection risk assessment. *Environment international*, *141*, 105794. https://doi.org/10.1016/j.envint.2020.105794
24. Butler, D., Mozsary, C., Meydan, C., Foox, J., Rosiene, J., Shaiber, A., Danko, D., Afshinnekoo, E., MacKay, M., Sedlazeck, F. J., Ivanov, N. A., Sierra, M., Pohle, D., Zietz, M., Gisladottir, U., Ramlall, V., Sholle, E. T., Schenck, E. J., Westover, C. D., Hassan, C., … Mason, C. E. (2020). Host, Viral, and Environmental Transcriptome Profiles of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). https://www.biorxiv.org/content/10.1101/2020.04.20.048066v1
25. Butler, D., Mozsary, C., Meydan, C., Foox, J., Rosiene, J., Shaiber, A., Danko, D., Afshinnekoo, E., MacKay, M., Sedlazeck, F. J., Ivanov, N. A., Sierra, M., Pohle, D., Zietz, M., Gisladottir, U., Ramlall, V., Sholle, E. T., Schenck, E. J., Westover, C. D., Hassan, C., … Mason, C. E. (2021). Shotgun transcriptome, spatial omics, and isothermal profiling of SARS-CoV-2 infection reveals unique host responses, viral diversification, and drug interactions. *Nature communications*, *12*(1), 1660. https://doi.org/10.1038/s41467-021-21361-7
26. Carter, C., Akrami, K., Hall, D., Smith, D., & Aronoff-Spencer, E. (2017). Lyophilized visually readable loop-mediated isothermal reverse transcriptase nucleic acid amplification test for detection Ebola Zaire RNA. *Journal of virological methods*, *244*, 32–38. https://doi.org/10.1016/j.jviromet.2017.02.013
27. Cevik, M., Tate, M., Lloyd, O., Maraolo, A. E., Schafers, J., & Ho, A. (2021). SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *The Lancet. Microbe*, *2*(1), e13–e22. https://doi.org/10.1016/S2666-5247(20)30172-5
28. Challener, C. (n.d.). *For Lyophilization, Excipients Really Do Matter*. BioPharm International. https://www.biopharminternational.com/view/lyophilization-excipients-really-do-matter.
29. Chander, Y., Koelbl, J., Puckett, J., Moser, M. J., Klingele, A. J., Liles, M. R., Carrias, A., Mead, D. A., & Schoenfeld, T. W. (2014). A novel thermostable polymerase for RNA and DNA loop-mediated isothermal amplification (LAMP). *Frontiers in microbiology*, *5*, 395. https://doi.org/10.3389/fmicb.2014.00395
30. Chaouch M. (2021). Loop-mediated isothermal amplification (LAMP): An effective molecular point-of-care technique for the rapid diagnosis of coronavirus SARS-CoV-2. *Reviews in medical virology*, e2215. Advance online publication. https://doi.org/10.1002/rmv.2215
31. Chen, H. W., & Ching, W. M. (2017). Evaluation of the stability of lyophilized loop-mediated isothermal amplification reagents for the detection of *Coxiella burnetii*. *Heliyon*, *3*(10), e00415. https://doi.org/10.1016/j.heliyon.2017.e00415
32. Cheng, Y., Niu, J., Zhang, Y., Huang, J., & Li, Q. (2006). Preparation of His-tagged armored RNA phage particles as a control for real-time reverse transcription-PCR detection of severe acute respiratory syndrome coronavirus. *Journal of clinical microbiology*, *44*(10), 3557–3561. https://doi.org/10.1128/JCM.00713-06

33. Chua, A. L., Elina, H. T., Lim, B. H., Yean, C. Y., Ravichandran, M., & Lalitha, P. (2011). Development of a dry reagent-based triplex PCR for the detection of toxigenic and non-toxigenic Vibrio cholerae. *Journal of medical microbiology*, *60*(Pt 4), 481–485. https://doi.org/10.1099/jmm.0.027433-0
34. Cimolai N. (2020). Potentially repurposing adamantanes for COVID-19. *Journal of medical virology*, *92*(6), 531–532. https://doi.org/10.1002/jmv.25752
35. Cody Carrell, Alyssa Kava, Michael Nguyen, Ruth Menger, Zarina Munshi, Zachary Call, Mark Nussbaum, & Charles Henry (2019). Beyond the lateral flow assay: A review of paper-based microfluidics. *Microelectronic Engineering, 206, 45-54.*
36. Contreras, J. L., Eisen, M., Ganz, A., Lemley, M., Molloy, J., Peters, D. M., & Tietze, F. (2020). Pledging intellectual property for COVID-19. *Nature biotechnology*, *38*(10), 1146–1149. https://doi.org/10.1038/s41587-020-0682-1
37. Cook, J., Aydin-Schmidt, B., González, I. J., Bell, D., Edlund, E., Nassor, M. H., Msellem, M., Ali, A., Abass, A. K., Mårtensson, A., & Björkman, A. (2015). Loop-mediated isothermal amplification (LAMP) for point-of-care detection of asymptomatic low-density malaria parasite carriers in Zanzibar. *Malaria journal*, *14*, 43. https://doi.org/10.1186/s12936-015-0573-y
38. Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M. L., Mulders, D. G., Haagmans, B. L., van der Veer, B., van den Brink, S., Wijsman, L., Goderski, G., Romette, J. L., Ellis, J., Zambon, M., Peiris, M., … Drosten, C. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin*, *25*(3), 2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045
39. Cox, H. S., Daniels, J. F., Muller, O., Nicol, M. P., Cox, V., van Cutsem, G., Moyo, S., De Azevedo, V., & Hughes, J. (2015). Impact of Decentralized Care and the Xpert MTB/RIF Test on Rifampicin-Resistant Tuberculosis Treatment Initiation in Khayelitsha, South Africa. *Open forum infectious diseases*, *2*(1), ofv014. https://doi.org/10.1093/ofid/ofv014
40. Danko D, Bezdan D, Afshin EE, Ahsanuddin S, Bhattacharya C, Butler DJ, Chng KR, Donnellan D, Hecht J, Jackson K, Kuchin K, Karasikov M, Lyons A, Mak L, Meleshko D, Mustafa H, Mutai B, Neches RY, Ng A, Nikolayeva O, Nikolayeva T, Png E, Ryon KA, Sanchez JL, Shaaban H, Sierra MA, Thomas D, Young B, Abudayyeh OO, Alicea J, Bhattacharyya M, Blekhman R, Castro-Nallar E, Cañas AM, Chatziefthimiou AD, Crawford RW, De Filippis F, Deng Y, Desnues C, Dias-Neto E, Dybwad M, Elhaik E, Ercolini D, Frolova A, Gankin D, Gootenberg JS, Graf AB, Green DC, Hajirasouliha I, Hastings JJA, Hernandez M, Iraola G, Jang S, Kahles A, Kelly FJ, Knights K, Kyrpides NC, Łabaj PP, Lee PKH, Leung MHY, Ljungdahl PO, Mason-Buck G, McGrath K, Meydan C, Mongodin EF, Moraes MO, Nagarajan N, Nieto-Caballero M, Noushmehr H, Oliveira M, Ossowski S, Osuolale OO, Özcan O, Paez-Espino D, Rascovan N, Richard H, Rätsch G, Schriml LM, Semmler T, Sezerman OU, Shi L, Shi T, Siam R, Song LH, Suzuki H, Court DS, Tighe SW, Tong X, Udekwu KI, Ugalde JA, Valentine B, Vassilev DI, Vayndorf EM, Velavan TP, Wu J, Zambrano MM, Zhu J, Zhu S, Mason CE; International MetaSUB Consortium. A global metagenomic map of urban microbiomes and antimicrobial resistance. *Cell*. 2021 May 22:S0092-8674(21)00585-7.
41. Dao Thi, V. L., Herbst, K., Boerner, K., Meurer, M., Kremer, L. P., Kirrmaier, D., Freistaedter, A., Papagiannidis, D., Galmozzi, C., Stanifer, M. L., Boulant, S., Klein, S., Chlanda, P., Khalid, D., Barreto Miranda, I., Schnitzler, P., Kräusslich, H. G., Knop, M., & Anders, S. (2020). A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. *Science translational medicine*, *12*(556), eabc7075. https://doi.org/10.1126/scitranslmed.abc7075
42. Dao Thi, V. L., Herbst, K., Boerner, K., Meurer, M., Kremer, L. P., Kirrmaier, D., Freistaedter, A., Papagiannidis, D., Galmozzi, C., Stanifer, M. L., Boulant, S., Klein, S., Chlanda, P., Khalid, D., Barreto Miranda, I., Schnitzler, P., Kräusslich, H. G., Knop, M., & Anders, S. (2020). A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. *Science translational medicine*, *12*(556), eabc7075. https://doi.org/10.1126/scitranslmed.abc7075
43. de Oliveira Coelho, B., Sanchuki, H., Zanette, D. L., Nardin, J. M., Morales, H., Fornazari, B., Aoki, M. N., & Blanes, L. (2021). Essential properties and pitfalls of colorimetric Reverse Transcription Loop-mediated Isothermal Amplification as a point-of-care test for SARS-CoV-2 diagnosis. *Molecular medicine (Cambridge, Mass.)*, *27*(1), 30. https://doi.org/10.1186/s10020-021-00289-0
44. de Oliveira Coelho, B., Sanchuki, H., Zanette, D. L., Nardin, J. M., Morales, H., Fornazari, B., Aoki, M. N., & Blanes, L. (2021). Essential properties and pitfalls of colorimetric Reverse Transcription Loop-mediated Isothermal Amplification as a point-of-care test for SARS-CoV-2 diagnosis. *Molecular medicine (Cambridge, Mass.)*, *27*(1), 30. https://doi.org/10.1186/s10020-021-00289-0

45. Diaz, L., Johnson, B., & Jenkins, D. (2021). Real-time optical analysis of a colorimetric LAMP assay for SARS-CoV-2 in saliva with a handheld instrument improves accuracy compared to endpoint assessment. *medRxiv*.
46. Dudley, D. M., Newman, C. M., Weiler, A. M., Ramuta, M. D., Shortreed, C. G., Heffron, A. S., Accola, M. A., Rehrauer, W. M., Friedrich, T. C., & O'Connor, D. H. (2020). Optimizing direct RT-LAMP to detect transmissible SARS-CoV-2 from primary nasopharyngeal swab samples. *PloS one*, *15*(12), e0244882. https://doi.org/10.1371/journal.pone.0244882
47. Eftekhari, A., Alipour, M., Chodari, L., Maleki Dizaj, S., Ardalan, M., Samiei, M., Sharifi, S., Zununi Vahed, S., Huseynova, I., Khalilov, R., Ahmadian, E., & Cucchiarini, M. (2021). A Comprehensive Review of Detection Methods for SARS-CoV-2. *Microorganisms*, *9*(2), 232. https://doi.org/10.3390/microorganisms9020232
48. Egerer, R., Edel, B., Löffler, B., Henke, A., & Rödel, J. (2021). Performance of the RT-LAMP-based eazyplex® SARS-CoV-2 as a novel rapid diagnostic test. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*, *138*, 104817. https://doi.org/10.1016/j.jcv.2021.104817
49. Elvira-González, L., Puchades, A. V., Carpino, C., Alfaro-Fernandez, A., Font-San-Ambrosio, M. I., Rubio, L., & Galipienso, L. (2017). Fast detection of Southern tomato virus by one-step transcription loop-mediated isothermal amplification (RT-LAMP). *Journal of virological methods*, *241*, 11–14. https://doi.org/10.1016/j.jviromet.2016.12.004
50. Farshidfar, N., & Hamedani, S. (2020). The Potential Role of Smartphone-Based Microfluidic Systems for Rapid Detection of COVID-19 Using Saliva Specimen. *Molecular diagnosis & therapy*, *24*(4), 371–373. https://doi.org/10.1007/s40291-020-00477-4
51. Farshidfar, N., & Hamedani, S. (2020). The Potential Role of Smartphone-Based Microfluidic Systems for Rapid Detection of COVID-19 Using Saliva Specimen. *Molecular diagnosis & therapy*, *24*(4), 371–373. https://doi.org/10.1007/s40291-020-00477-4
52. Fei, Z., Wei, R., Cheng, C., & Xiao, P. (2021). A Novel Approach to the Bioluminescent Detection of the SARS-CoV-2 ORF1ab Gene by Coupling Isothermal RNA Reverse Transcription Amplification with a Digital PCR Approach. *International journal of molecular sciences*, *22*(3), 1017. https://doi.org/10.3390/ijms22031017
53. Filipić, A., Dobnik, D., Tušek Žnidarič, M., Žegura, B., Štern, A., Primc, G., Mozetič, M., Ravnikar, M., Žel, J., & Gutierrez Aguirre, I. (2021). Inactivation of Pepper Mild Mottle Virus in Water by Cold Atmospheric Plasma. *Frontiers in microbiology*, *12*, 618209. https://doi.org/10.3389/fmicb.2021.618209
54. Finkel, Y., Mizrahi, O., Nachshon, A., Weingarten-Gabbay, S., Morgenstern, D., Yahalom-Ronen, Y., Tamir, H., Achdout, H., Stein, D., Israeli, O., Beth-Din, A., Melamed, S., Weiss, S., Israely, T., Paran, N., Schwartz, M., & Stern-Ginossar, N. (2021). The coding capacity of SARS-CoV-2. *Nature*, *589*(7840), 125–130. https://doi.org/10.1038/s41586-020-2739-1
55. Flynn, M., Snitser, O., Flynn, J., Green, S., Yelin, I., Szwarcwort-Cohen, M., Kishony, R., & Elowitz, M. (2020). A simple direct RT-LAMP SARS-CoV-2 saliva diagnostic. *medRxiv*.
56. Foox J, Tighe SW, Nicolet CM, Zook JM, Herbert ZT, Warner D, Grills GS, Levy S, Xiang J, Alonzo A, Schroth GP, Farmerie W, Baldwin DA, Mason CE. Multi-Platform Assessment of DNA Sequencing Performance using Human and Bacterial Reference Genomes in the ABRF Next-Generation Sequencing Study. *Nature Biotechnology*. 2021.
57. Fowler, J., Hill, S., Levin, R., & Obradovich, N. (2020). The Effect of Stay-at-Home Orders on COVID-19 Cases and Fatalities in the United States. *medRxiv*.
58. Fowler, V. L., Armson, B., Gonzales, J. L., Wise, E. L., Howson, E., Vincent-Mistiaen, Z., Fouch, S., Maltby, C. J., Grippon, S., Munro, S., Jones, L., Holmes, T., Tillyer, C., Elwell, J., Sowood, A., de Peyer, O., Dixon, S., Hatcher, T., Patrick, H., Laxman, S., … Kidd, S. P. (2021). A highly effective reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the rapid detection of SARS-CoV-2 infection. *The Journal of infection*, *82*(1), 117–125. https://doi.org/10.1016/j.jinf.2020.10.039
59. Francois, P., Tangomo, M., Hibbs, J., Bonetti, E. J., Boehme, C. C., Notomi, T., Perkins, M. D., & Schrenzel, J. (2011). Robustness of a loop-mediated isothermal amplification reaction for diagnostic applications. *FEMS immunology and medical microbiology*, *62*(1), 41–48. https://doi.org/10.1111/j.1574-695X.2011.00785.x
60. Ganguli, A., Mostafa, A., Berger, J., Aydin, M., Sun, F., Valera, E., Cunningham, B. T., King, W. P., & Bashir, R. (2020). Rapid Isothermal Amplification and Portable Detection System for SARS-CoV-2. *bioRxiv : the preprint server for biology*, 2020.05.21.108381. https://doi.org/10.1101/2020.05.21.108381
61. García-Bernalt Diego, J., Fernández-Soto, P., Crego-Vicente, B., Alonso-Castrillejo, S., Febrer-Sendra, B., Gómez-Sánchez, A., Vicente, B., López-Abán, J., & Muro, A. (2019). Progress in loop-mediated isothermal amplification assay for detection of Schistosoma mansoni DNA: towards a ready-to-use test. *Scientific reports*, *9*(1), 14744. https://doi.org/10.1038/s41598-019-51342-2

62. García-Bernalt Diego, J., Fernández-Soto, P., Domínguez-Gil, M., Belhassen-García, M., Bellido, J., & Muro, A. (2021). A Simple, Affordable, Rapid, Stabilized, Colorimetric, Versatile RT-LAMP Assay to Detect SARS-CoV-2. *Diagnostics (Basel, Switzerland)*, *11*(3), 438. https://doi.org/10.3390/diagnostics11030438
63. García-Bernalt Diego, J., Fernández-Soto, P., Febrer-Sendra, B., Crego-Vicente, B., & Muro, A. (2021). Loop-Mediated Isothermal Amplification in Schistosomiasis. *Journal of clinical medicine*, *10*(3), 511. https://doi.org/10.3390/jcm10030511
64. Garcia-Venzor, A., Rueda-Zarazua, B., Marquez-Garcia, E., Maldonado, V., Moncada-Morales, A., Olivera, H., Lopez, I., Zuñiga, J., & Melendez-Zajgla, J. (2021). SARS-CoV-2 Direct Detection Without RNA Isolation With Loop-Mediated Isothermal Amplification (LAMP) and CRISPR-Cas12. *Frontiers in medicine*, *8*, 627679. https://doi.org/10.3389/fmed.2021.627679
65. Garneret, P., Coz, E., Martin, E., Manuguerra, J. C., Brient-Litzler, E., Enouf, V., González Obando, D. F., Olivo-Marin, J. C., Monti, F., van der Werf, S., Vanhomwegen, J., & Tabeling, P. (2021). Performing point-of-care molecular testing for SARS-CoV-2 with RNA extraction and isothermal amplification. *PloS one*, *16*(1), e0243712. https://doi.org/10.1371/journal.pone.0243712
66. Gonçalves, D., Hooker, D. J., Dong, Y., Baran, N., Kyrylos, P., Iturbe-Ormaetxe, I., Simmons, C. P., & O'Neill, S. L. (2019). Detecting wMel Wolbachia in field-collected Aedes aegypti mosquitoes using loop-mediated isothermal amplification (LAMP). *Parasites & vectors*, *12*(1), 404. https://doi.org/10.1186/s13071-019-3666-6
67. Gong, M. M., & Sinton, D. (2017). Turning the Page: Advancing Paper-Based Microfluidics for Broad Diagnostic Application. *Chemical reviews*, *117*(12), 8447–8480. https://doi.org/10.1021/acs.chemrev.7b00024
68. González-González, E., Lara-Mayorga, I., Rodríguez-Sánchez, I., León, F.d., García-Rubio, A., Garciaméndez-Mijares, C., Guerra-Alvarez, G., García-Martínez, G., Aguayo-Hernández, J., Márquez-García, E., Zhang, Y., Martínez-Chapa, S., Zúñiga, J., Santiago, G.d., & Alvarez, M.. (2020). Scaling diagnostics in times of COVID-19: Colorimetric Loop-mediated Isothermal Amplification (LAMP) assisted by a 3D-printed incubator for cost-effective and scalable detection of SARS-CoV-2.
69. Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, Dy AJ, Joung J, Verdine V, Donghia N, Daringer NM, Freije CA, Myhrvold C, Bhattacharyya RP, Livny J, Regev A, Koonin EV, Hung DT, Sabeti PC, Collins JJ, Zhang F. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science. 2017 Apr 28;356(6336):438-442. doi: 10.1126/science.aam9321. Epub 2017 Apr 13. PMID: 28408723; PMCID: PMC5526198.
70. Goto, M., Honda, E., Ogura, A., Nomoto, A., & Hanaki, K. (2009). Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxy naphthol blue. *BioTechniques*, *46*(3), 167–172. https://doi.org/10.2144/000113072
71. Guo, L., Sun, X., Wang, X., Liang, C., Jiang, H., Gao, Q., Dai, M., Qu, B., Fang, S., Mao, Y., Chen, Y., Feng, G., Gu, Q., Wang, R. R., Zhou, Q., & Li, W. (2020). SARS-CoV-2 detection with CRISPR diagnostics. *Cell discovery*, *6*, 34. https://doi.org/10.1038/s41421-020-0174-y
72. Gupta, S., Parker, J., Smits, S., Underwood, J., & Dolwani, S. (2020). Persistent viral shedding of SARS-CoV-2 in faeces - a rapid review. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland*, *22*(6), 611–620. https://doi.org/10.1111/codi.15138.
73. Haibe-Kains B, Adam GA, Hosny A, Khodakarami F; Massive Analysis Quality Control (MAQC) Society Board of Directors, Waldron L, Wang B, McIntosh C, Goldenberg A, Kundaje A, Greene CS, Broderick T, Hoffman MM, Leek JT, Korthauer K, Huber W, Brazma A, Pineau J, Tibshirani R, Hastie T, Ioannidis JPA, Quackenbush J, Aerts HJWL. Transparency and reproducibility in artificial intelligence. *Nature*. 2020 Oct;586(7829):E14-E16.
74. Hardinge, P., Kiddle, G., Tisi, L., & Murray, J. (2018). Optimised LAMP allows single copy detection of 35Sp and NOSt in transgenic maize using Bioluminescent Assay in Real Time (BART). *Scientific reports*, *8*(1), 17590. https://doi.org/10.1038/s41598-018-36207-4
75. Hasell, J., Mathieu, E., Beltekian, D., Macdonald, B., Giattino, C., Ortiz-Ospina, E., Roser, M., & Ritchie, H. (2020). A cross-country database of COVID-19 testing. *Scientific data*, *7*(1), 345. https://doi.org/10.1038/s41597-020-00688-8
76. Helgouach, N., Champigneux, P., Schneider, F., Molina, L., Espeut, J., Alali, M., Baptiste, J., Cardeur, L., Dubuc, B., Foulongne, V., Galtier, F., Makinson, A., Marin, G., Picot, M.C., Prieux-Lejeune, A., Quenot, M., Robles, F., Salvetat, N., Vetter, D., Reynes, J., & Molina, F. (2020). EasyCOV : LAMP based rapid detection of SARS-CoV-2 in saliva. *medRxiv*.
77. Howson, E., Kidd, S. P., Armson, B., Goring, A., Sawyer, J., Cassar, C., Cross, D., Lewis, T., Hockey, J., Rivers, S., Cawthraw, S., Banyard, A., Anderson, P., Rahou, S., Andreou, M., Morant, N., Clark, D., Walsh, C., Laxman, S., Houghton, R., … Fowler, V. L. (2021). Preliminary optimisation of a simplified sample preparation method to permit

direct detection of SARS-CoV-2 within saliva samples using reverse-transcription loop-mediated isothermal amplification (RT-LAMP). *Journal of virological methods*, *289*, 114048. https://doi.org/10.1016/j.jviromet.2020.114048

78. Howson, E., Kidd, S. P., Armson, B., Goring, A., Sawyer, J., Cassar, C., Cross, D., Lewis, T., Hockey, J., Rivers, S., Cawthraw, S., Banyard, A., Anderson, P., Rahou, S., Andreou, M., Morant, N., Clark, D., Walsh, C., Laxman, S., Houghton, R., … Fowler, V. L. (2021). Preliminary optimisation of a simplified sample preparation method to permit direct detection of SARS-CoV-2 within saliva samples using reverse-transcription loop-mediated isothermal amplification (RT-LAMP). *Journal of virological methods*, *289*, 114048. https://doi.org/10.1016/j.jviromet.2020.114048
79. Hu, B., Guo, H., Zhou, P., & Shi, Z. L. (2021). Characteristics of SARS-CoV-2 and COVID-19. *Nature reviews. Microbiology*, *19*(3), 141–154. https://doi.org/10.1038/s41579-020-00459-7
80. Hu, C., Kalsi, S., Zeimpekis, I., Sun, K., Ashburn, P., Turner, C., Sutton, J. M., & Morgan, H. (2017). Ultra-fast electronic detection of antimicrobial resistance genes using isothermal amplification and Thin Film Transistor sensors. *Biosensors & bioelectronics*, *96*, 281–287. https://doi.org/10.1016/j.bios.2017.05.016
81. Hu, F., Li, J., Zhang, Z., Li, M., Zhao, S., Li, Z., & Peng, N. (2020). Smartphone-Based Droplet Digital LAMP Device with Rapid Nucleic Acid Isolation for Highly Sensitive Point-of-Care Detection. *Analytical chemistry*, *92*(2), 2258–2265. https://doi.org/10.1021/acs.analchem.9b04967
82. Huang, X., Lin, X., Urmann, K., Li, L., Xie, X., Jiang, S., & Hoffmann, M. R. (2018). Smartphone-Based in-Gel Loop-Mediated Isothermal Amplification (gLAMP) System Enables Rapid Coliphage MS2 Quantification in Environmental Waters. *Environmental science & technology*, *52*(11), 6399–6407. https://doi.org/10.1021/acs.est.8b00241
83. Ignatov, K. B., Barsova, E. V., Fradkov, A. F., Blagodatskikh, K. A., Kramarova, T. V., & Kramarov, V. M. (2014). A strong strand displacement activity of thermostable DNA polymerase markedly improves the results of DNA amplification. *BioTechniques*, *57*(2), 81–87. https://doi.org/10.2144/000114198
84. James, P., Stoddart, D., Harrington, E., Beaulaurier, J., Ly, L., Reid, S., Turner, D., & Juul, S. (2020). LamPORE: rapid, accurate and highly scalable molecular screening for SARS-CoV-2 infection, based on nanopore sequencing. *medRxiv*.
85. Jani, I. V., & Peter, T. F. (2013). How point-of-care testing could drive innovation in global health. *The New England journal of medicine*, *368*(24), 2319–2324. https://doi.org/10.1056/NEJMsb1214197
86. Janíková, M., Hodosy, J., Boor, P., Klempa, B., & Celec, P. (2021). Loop-mediated isothermal amplification for the detection of SARS-CoV-2 in saliva. *Microbial biotechnology*, *14*(1), 307–316. https://doi.org/10.1111/1751-7915.13737
87. Jia, B., Li, X., Liu, W., Lu, C., Lu, X., Ma, L., Li, Y. Y., & Wei, C. (2019). GLAPD: Whole Genome Based LAMP Primer Design for a Set of Target Genomes. *Frontiers in microbiology*, *10*, 2860. https://doi.org/10.3389/fmicb.2019.02860
88. Jiang, L., Mancuso, M., Lu, Z., Akar, G., Cesarman, E., & Erickson, D. (2014). Solar thermal polymerase chain reaction for smartphone-assisted molecular diagnostics. *Scientific reports*, *4*, 4137. https://doi.org/10.1038/srep04137
89. Johnson, Mary (2013). **Detergents: Triton X-100, Tween-20, and More** MATER METHODS 2013;3:163
90. Jureka, A. S., Silvas, J. A., & Basler, C. F. (2020). Propagation, Inactivation, and Safety Testing of SARS-CoV-2. *Viruses*, *12*(6), 622. https://doi.org/10.3390/v12060622
91. Jureka, A. S., Silvas, J. A., & Basler, C. F. (2020). Propagation, Inactivation, and Safety Testing of SARS-CoV-2. *Viruses*, *12*(6), 622. https://doi.org/10.3390/v12060622
92. Kaarj, K., Akarapipad, P., & Yoon, J. Y. (2018). Simpler, Faster, and Sensitive Zika Virus Assay Using Smartphone Detection of Loop-mediated Isothermal Amplification on Paper Microfluidic Chips. *Scientific reports*, *8*(1), 12438. https://doi.org/10.1038/s41598-018-30797-9
93. Kampf, G., Todt, D., Pfaender, S., & Steinmann, E. (2020). Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *The Journal of hospital infection*, *104*(3), 246–251. https://doi.org/10.1016/j.jhin.2020.01.022
94. Kaneko, H., Kawana, T., Fukushima, E., & Suzutani, T. (2007). Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. *Journal of biochemical and biophysical methods*, *70*(3), 499–501. https://doi.org/10.1016/j.jbbm.2006.08.008
95. Kellner, M., Ross, J., Schnabl, J., Dekens, M., Heinen, R., Grishkovskaya, I., Bauer, B., Stadlmann, J., Menéndez-Arias, L., Fritsche-Polanz, R., Traugott, M., Seitz, T., Zoufaly, A., Födinger, M., Wenisch, C., Zuber, J., , Pauli, A., & Brennecke, J. (2020). A rapid, highly sensitive and open-access SARS-CoV-2 detection assay for laboratory and home testing. *bioRxiv*.

96. Khorosheva, E. M., Karymov, M. A., Selck, D. A., & Ismagilov, R. F. (2016). Lack of correlation between reaction speed and analytical sensitivity in isothermal amplification reveals the value of digital methods for optimization: validation using digital real-time RT-LAMP. *Nucleic acids research*, *44*(2), e10. https://doi.org/10.1093/nar/gkv877
97. Kiefer, J. R., Mao, C., Hansen, C. J., Basehore, S. L., Hogrefe, H. H., Braman, J. C., & Beese, L. S. (1997). Crystal structure of a thermostable Bacillus DNA polymerase I large fragment at 2.1 A resolution. *Structure (London, England : 1993)*, *5*(1), 95–108. https://doi.org/10.1016/s0969-2126(97)00169-x
98. Kim, E. M., Jeon, H. S., Kim, J. J., Shin, Y. K., Lee, Y. J., Yeo, S. G., & Park, C. K. (2016). Uracil-DNA glycosylase-treated reverse transcription loop-mediated isothermal amplification for rapid detection of avian influenza virus preventing carry-over contamination. *Journal of veterinary science*, *17*(3), 421–425. https://doi.org/10.4142/jvs.2016.17.3.421
99. Kim, J. M., Kim, H. M., Lee, E. J., Jo, H. J., Yoon, Y., Lee, N. J., Son, J., Lee, Y. J., Kim, M. S., Lee, Y. P., Chae, S. J., Park, K. R., Cho, S. R., Park, S., Kim, S. J., Wang, E., Woo, S., Lim, A., Park, S. J., Jang, J., … Yoo, C. K. (2020). Detection and Isolation of SARS-CoV-2 in Serum, Urine, and Stool Specimens of COVID-19 Patients from the Republic of Korea. *Osong public health and research perspectives*, *11*(3), 112–117. https://doi.org/10.24171/j.phrp.2020.11.3.02
100. Klein, S., Müller, T. G., Khalid, D., Sonntag-Buck, V., Heuser, A. M., Glass, B., Meurer, M., Morales, I., Schillak, A., Freistaedter, A., Ambiel, I., Winter, S. L., Zimmermann, L., Naumoska, T., Bubeck, F., Kirrmaier, D., Ullrich, S., Barreto Miranda, I., Anders, S., Grimm, D., … Chlanda, P. (2020). SARS-CoV-2 RNA Extraction Using Magnetic Beads for Rapid Large-Scale Testing by RT-qPCR and RT-LAMP. *Viruses*, *12*(8), 863. https://doi.org/10.3390/v12080863
101. La Scola, B., Le Bideau, M., Andreani, J., Hoang, V. T., Grimaldier, C., Colson, P., Gautret, P., & Raoult, D. (2020). Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology*, *39*(6), 1059–1061. https://doi.org/10.1007/s10096-020-03913-9
102. LaBarre, P., Hawkins, K. R., Gerlach, J., Wilmoth, J., Beddoe, A., Singleton, J., Boyle, D., & Weigl, B. (2011). A simple, inexpensive device for nucleic acid amplification without electricity-toward instrument-free molecular diagnostics in low-resource settings. *PloS one*, *6*(5), e19738. https://doi.org/10.1371/journal.pone.0019738
103. Lalli, M. A., Langmade, J. S., Chen, X., Fronick, C. C., Sawyer, C. S., Burcea, L. C., Wilkinson, M. N., Fulton, R. S., Heinz, M., Buchser, W. J., Head, R. D., Mitra, R. D., & Milbrandt, J. (2021). Rapid and Extraction-Free Detection of SARS-CoV-2 from Saliva by Colorimetric Reverse-Transcription Loop-Mediated Isothermal Amplification. *Clinical chemistry*, *67*(2), 415–424. https://doi.org/10.1093/clinchem/hvaa267
104. Lamb, L. E., Bartolone, S. N., Ward, E., & Chancellor, M. B. (2020). Rapid detection of novel coronavirus/Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by reverse transcription-loop-mediated isothermal amplification. *PloS one*, *15*(6), e0234682. https://doi.org/10.1371/journal.pone.0234682
105. Larremore, D., Wilder, B., Lester, E., Shehata, S., Burke, J., Hay, J., Tambe, M., Mina, M., & Parker, R. (2020). Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance. *medRxiv*.
106. Lau, Y. L., Ismail, I., Mustapa, N. I., Lai, M. Y., Tuan Soh, T. S., Hassan, A., Peariasamy, K. M., Lee, Y. L., Chong, Y. M., Sam, I. C., & Goh, P. P. (2020). Real-time reverse transcription loop-mediated isothermal amplification for rapid detection of SARS-CoV-2. *PeerJ*, *8*, e9278. https://doi.org/10.7717/peerj.9278
107. Li, Ethan & Larson, Adam & Kothari, Anestha & Prakash, Manu. (2020). Handyfuge-LAMP: low-cost and electricity-free centrifugation for isothermal SARS-CoV-2 detection in saliva. 10.1101/2020.06.30.20143255.
108. Li, J., Macdonald, J., & von Stetten, F. (2018). Review: a comprehensive summary of a decade development of the recombinase polymerase amplification. *The Analyst*, *144*(1), 31–67. https://doi.org/10.1039/c8an01621f
109. Li S, Tighe SW, Nicolet CM, Grove D, Levy S, Farmerie W, Viale A, Wright C, Schweitzer PA , Gao, Kim D, Boland J, Hicks B, Kim R, Chhangawala S, Jafari D, Raghavachari N, Gandara J, Garcia-Reyero N, Hendrickson C, Roberson D, Rosenfeld JA, Smith T, Underwood JG, Wang M, Zumbo P, Baldwin DA, Grills GS, Mason CE. "Multi-Platform Assessment of Transcriptome Profiling Using RNA-Seq in the ABRF Next Generation Sequencing Study." *Nature Biotechnology*. 2014 Sep;32(9):915-25.
110. Liao, S. C., Peng, J., Mauk, M. G., Awasthi, S., Song, J., Friedman, H., Bau, H. H., & Liu, C. (2016). Smart Cup: A Minimally-Instrumented, Smartphone-Based Point-of-Care Molecular Diagnostic Device. *Sensors and actuators. B, Chemical*, *229*, 232–238. https://doi.org/10.1016/j.snb.2016.01.073
111. Lin, X., Huang, X., Urmann, K., Xie, X., & Hoffmann, M. R. (2019). Digital Loop-Mediated Isothermal Amplification on a Commercial Membrane. *ACS sensors*, *4*(1), 242–249. https://doi.org/10.1021/acssensors.8b01419

112. Liu H, Begik O, Lucas MC, Ramirez JM, Mason CE, Wiener D, Schwartz S, Mattick JS, Smith MA, Novoa EM. Accurate detection of m6A RNA modifications in native RNA sequences. *Nature Communications*. 2019 Sep 9;10(1):4079.
113. Loveday, E. K., Hain, K. S., Kochetkova, I., Hedges, J. F., Robison, A., Snyder, D. T., Brumfield, S. K., Young, M. J., Jutila, M. A., Chang, C. B., & Taylor, M. P. (2021). Effect of Inactivation Methods on SARS-CoV-2 Virion Protein and Structure. *Viruses*, *13*(4), 562. https://doi.org/10.3390/v13040562
114. Ma, J., Su, C., Hu, S., Chen, Y., Shu, Y., Yue, D., Zhang, B., Qi, Z., Li, S., Wang, X., Kuang, Y., & Cheng, P. (2020). The Effect of Residual Triton X-100 on Structural Stability and Infection Activity of Adenovirus Particles. *Molecular therapy. Methods & clinical development*, *19*, 35–46. https://doi.org/10.1016/j.omtm.2020.08.013
115. MacKay, M. J., Hooker, A. C., Afshinnekoo, E., Salit, M., Kelly, J., Feldstein, J. V., Haft, N., Schenkel, D., Nambi, S., Cai, Y., Zhang, F., Church, G., Dai, J., Wang, C. L., Levy, S., Huber, J., Ji, H. P., Kriegel, A., Wyllie, A. L., & Mason, C. E. (2020). The COVID-19 XPRIZE and the need for scalable, fast, and widespread testing. *Nature biotechnology*, *38*(9), 1021–1024. https://doi.org/10.1038/s41587-020-0655-4
116. Maia Chagas, A., Molloy, J. C., Prieto-Godino, L. L., & Baden, T. (2020). Leveraging open hardware to alleviate the burden of COVID-19 on global health systems. *PLoS biology*, *18*(4), e3000730. https://doi.org/10.1371/journal.pbio.3000730
117. Maranhao, A., Bhadra, S., Paik, I., Walker, D., & Ellington, A. (2020). An improved and readily available version of Bst DNA Polymerase for LAMP, and applications to COVID-19 diagnostics. *medRxiv*.
118. Martineau, A. R., Jolliffe, D. A., Hooper, R. L., Greenberg, L., Aloia, J. F., Bergman, P., Dubnov-Raz, G., Esposito, S., Ganmaa, D., Ginde, A. A., Goodall, E. C., Grant, C. C., Griffiths, C. J., Janssens, W., Laaksi, I., Manaseki-Holland, S., Mauger, D., Murdoch, D. R., Neale, R., Rees, J. R., … Camargo, C. A., Jr (2017). Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. *BMJ (Clinical research ed.)*, *356*, i6583. https://doi.org/10.1136/bmj.i6583
119. Mary Natoli, Kathryn Kundrod, Megan Chang, Chelsey Smith, Sai Paul, Karen Eldin, Keyur Patel, Ellen Baker, Kathleen Schmeler, & Rebecca Richards-Kortum (2021). Reverse transcription loop-mediated isothermal amplification (RT-LAMP) for point-of-care detection of SARS-CoV-2: a clinical study to evaluate agreement with RT-qPCR. *The Lancet Global Health, 9, S3*.
120. Mason CE, Afshinnekoo E, Tighe S, Wu S Levy S. "International Standards for Genomes, Transcriptomes, and Metagenomes." *Journal of Biomolecular Techniques.* 2017 Apr;28(1):8-18.
121. Mason, M., & Botella, J. (2019). A simple, robust and equipment-free DNA amplification readout in less than 30 seconds. *RSC Adv., 9, 24440-24450.*
122. Mawaddah, A., Gendeh, H. S., Lum, S. G., & Marina, M. B. (2020). Upper respiratory tract sampling in COVID-19. *The Malaysian journal of pathology*, *42*(1), 23–35.
123. McIntyre ABR, Rizzardi L, Yu AM, Alexander N, Rosen GL, Botkin DJ, Stahl SS, John KK, Castro-Wallace SL, McGrath K, Burton AS, Feinberg AP, Mason CE. "Nanopore Sequencing in Microgravity." *Nature Partner Journals (npj) Microgravity. 2, 2016:16035.*
124. McIntyre ABR, Alexander N, Grigorev K, Bezdan D, Sichtig H, Chiu CY, Mason CE. Single-molecule sequencing detection of N6-methyladenine in microbial reference materials. Nature Communications. 2019 Feb 4;10(1):579.
125. Milligan, J. N., Shroff, R., Garry, D. J., & Ellington, A. D. (2018). Evolution of a Thermophilic Strand-Displacing Polymerase Using High-Temperature Isothermal Compartmentalized Self-Replication. *Biochemistry*, *57*(31), 4607–4619. https://doi.org/10.1021/acs.biochem.8b00200
126. Mina, M. J., Parker, R., & Larremore, D. B. (2020). Rethinking Covid-19 Test Sensitivity - A Strategy for Containment. *The New England journal of medicine*, *383*(22), e120. https://doi.org/10.1056/NEJMp2025631
127. Miyamoto, S., Sano, S., Takahashi, K., & Jikihara, T. (2015). Method for colorimetric detection of double-stranded nucleic acid using leuco triphenylmethane dyes. *Analytical biochemistry*, *473*, 28–33. https://doi.org/10.1016/j.ab.2014.12.016
128. Moehling, T. J., Choi, G., Dugan, L. C., Salit, M., & Meagher, R. J. (2021). LAMP Diagnostics at the Point-of-Care: Emerging Trends and Perspectives for the Developer Community. *Expert review of molecular diagnostics*, *21*(1), 43–61. https://doi.org/10.1080/14737159.2021.1873769
129. Mohon, A. N., Getie, S., Jahan, N., Alam, M. S., & Pillai, D. R. (2019). Ultrasensitive loop mediated isothermal amplification (US-LAMP) to detect malaria for elimination. *Malaria journal*, *18*(1), 350. https://doi.org/10.1186/s12936-019-2979-4

130. Mori, Y., Nagamine, K., Tomita, N., & Notomi, T. (2001). Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochemical and biophysical research communications*, *289*(1), 150–154. https://doi.org/10.1006/bbrc.2001.5921
131. Mulberry, G., White, K. A., Vaidya, M., Sugaya, K., & Kim, B. N. (2017). 3D printing and milling a real-time PCR device for infectious disease diagnostics. *PloS one*, *12*(6), e0179133. https://doi.org/10.1371/journal.pone.0179133
132. Nagamine, K., Hase, T., & Notomi, T. (2002). Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Molecular and cellular probes*, *16*(3), 223–229. https://doi.org/10.1006/mcpr.2002.0415
133. Nagamine, K., Hase, T., & Notomi, T. (2002). Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Molecular and cellular probes*, *16*(3), 223–229. https://doi.org/10.1006/mcpr.2002.0415
134. Nagura-Ikeda, M., Imai, K., Tabata, S., Miyoshi, K., Murahara, N., Mizuno, T., Horiuchi, M., Kato, K., Imoto, Y., Iwata, M., Mimura, S., Ito, T., Tamura, K., & Kato, Y. (2020). Clinical Evaluation of Self-Collected Saliva by Quantitative Reverse Transcription-PCR (RT-qPCR), Direct RT-qPCR, Reverse Transcription-Loop-Mediated Isothermal Amplification, and a Rapid Antigen Test To Diagnose COVID-19. *Journal of clinical microbiology*, *58*(9), e01438-20. https://doi.org/10.1128/JCM.01438-20
135. Nagura-Ikeda, M., Imai, K., Tabata, S., Miyoshi, K., Murahara, N., Mizuno, T., Horiuchi, M., Kato, K., Imoto, Y., Iwata, M., Mimura, S., Ito, T., Tamura, K., & Kato, Y. (2020). Clinical Evaluation of Self-Collected Saliva by Quantitative Reverse Transcription-PCR (RT-qPCR), Direct RT-qPCR, Reverse Transcription-Loop-Mediated Isothermal Amplification, and a Rapid Antigen Test To Diagnose COVID-19. *Journal of clinical microbiology*, *58*(9), e01438-20. https://doi.org/10.1128/JCM.01438-20
136. Nangle SN, Wolfson MY, Hartsough L, Ma N, et al. The Case for Biotechnology on Mars. *Nature Biotechnology*. 2020 Apr;38(4):401-407.
137. Nawattanapaiboon, K., Pasomsub, E., Prombun, P., Wongbunmak, A., Jenjitwanich, A., Mahasupachai, P., Vetcho, P., Chayrach, C., Manatjaroenlap, N., Samphaongern, C., Watthanachockchai, T., Leedorkmai, P., Manopwisedjaroen, S., Akkarawongsapat, R., Thitithanyanont, A., Phanchana, M., Panbangred, W., Chauvatcharin, S., & Srikhirin, T. (2021). Colorimetric reverse transcription loop-mediated isothermal amplification (RT-LAMP) as a visual diagnostic platform for the detection of the emerging coronavirus SARS-CoV-2. *Analyst, 146, 471-477*.
138. Newman, C., Ramuta, M., McLaughlin, M., Wiseman, R., Karl, J., Dudley, D., Stauss, M., Maddox, R., Weiler, A., Bliss, M., Fauser, K., Haddock, L., Shortreed, C., Haj, A., Accola, M., Heffron, A., Bussan, H., Reynolds, M., Harwood, O., Moriarty, R., Stewart, L., Crooks, C., Prall, T., Neumann, E., Somsen, E., Burmeister, C., Hall, K., Rehrauer, W., Friedrich, T., O'Connor, S., & O'Connor, D. (2021). Initial evaluation of a mobile SARS-CoV-2 RT-LAMP testing strategy. *medRxiv*.
139. Ng DL, Granados AC, Santos YA, Servellita V, Goldgof GM, Meydan C, Sotomayor-Gonzalez A, Levine AG, Balcerek J, Han LM, Akagi N, *et al*. A diagnostic host response biosignature for COVID-19 from RNA profiling of nasal swabs and blood. *Science Advances*. 2021 Feb 3;7(6):eabe5984.
140. Nguyen, H. V., Nguyen, V. D., Liu, F., & Seo, T. S. (2020). An Integrated Smartphone-Based Genetic Analyzer for Qualitative and Quantitative Pathogen Detection. *ACS omega*, *5*(35), 22208–22214. https://doi.org/10.1021/acsomega.0c02317
141. Niemz, A., Ferguson, T. M., & Boyle, D. S. (2011). Point-of-care nucleic acid testing for infectious diseases. *Trends in biotechnology*, *29*(5), 240–250. https://doi.org/10.1016/j.tibtech.2011.01.007
142. Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic acids research*, *28*(12), E63. https://doi.org/10.1093/nar/28.12.e63
143. Novoa EM, Mason CE and Mattick JS. Charting the unknown human epitranscriptome. *Nature Reviews Molecular Cell Biology*. Nat Rev Mol Cell Biol. 2017 Jun;18(6):339-340.
144. Oliveira, K., Estrela, P., Mendes, G., Santos, C., Silveira-Lacerda, E., & Duarte, G. (2021). Rapid molecular diagnostics of COVID-19 by RT-LAMP in a centrifugal polystyrene-toner based microdevice with end-point visual detection. *Analyst, 146, 1178-1187*.
145. Oscorbin, I. P., Belousova, E. A., Zakabunin, A. I., Boyarskikh, U. A., & Filipenko, M. L. (2016). Comparison of fluorescent intercalating dyes for quantitative loop-mediated isothermal amplification (qLAMP). *BioTechniques*, *61*(1), 20–25. https://doi.org/10.2144/000114432
146. Pack, Todd Denison and Deng, Xiaokang (2008). Stable reagents and kits useful in loop-mediated isothermal amplification (LAMP)**. US20080182312A1. US patent and trademark office.** https://patents.google.com/patent/US20080182312A1/en

147. Papadakis, G., Pantazis, A., Fikas, N., Chatziioannidou, S., Michaelidou, K., Pogka, V., Megariti, M., Vardaki, M., Giarentis, K., Heaney, J., Nastouli, E., Karamitros, T., Mentis, A., Agelaki, S., & Gizeli, E. (2020). Real-time colorimetric LAMP methodology for quantitative nucleic acids detection at the point-of-care. *bioRxiv*.
148. Parker CW, Singh N, Tighe S, Blachowicz A, Wood JM, Seuylemezian A, Vaishampayan P, Urbaniak C, Hendrickson R, Laaguiby P, Clark K, Clement BG, O'Hara NB, Couto-Rodriguez M, Bezdan D, Mason CE, Venkateswaran K. End-to-End Protocol for the Detection of SARS-CoV-2 from Built Environments. *mSystems*. 2020 Oct 6;5(5):e00771-20.
149. Pastorino, B., Touret, F., Gilles, M., Luciani, L., de Lamballerie, X., & Charrel, R. N. (2020). Evaluation of Chemical Protocols for Inactivating SARS-CoV-2 Infectious Samples. *Viruses*, *12*(6), 624. https://doi.org/10.3390/v12060624
150. Peng, L., Liu, J., Xu, W., Luo, Q., Chen, D., Lei, Z., Huang, Z., Li, X., Deng, K., Lin, B., & Gao, Z. (2020). SARS-CoV-2 can be detected in urine, blood, anal swabs, and oropharyngeal swabs specimens. *Journal of medical virology*, *92*(9), 1676–1680. https://doi.org/10.1002/jmv.25936
151. Phang, S. M., Teo, C. Y., Lo, E., & Wong, V. W. (1995). Cloning and complete sequence of the DNA polymerase-encoding gene (BstpolI) and characterisation of the Klenow-like fragment from Bacillus stearothermophilus. *Gene*, *163*(1), 65–68. https://doi.org/10.1016/0378-1119(95)00387-l
152. Piepenburg, O., Williams, C. H., Stemple, D. L., & Armes, N. A. (2006). DNA detection using recombination proteins. *PLoS biology*, *4*(7), e204. https://doi.org/10.1371/journal.pbio.0040204
153. Pilcher, C. D., Westreich, D., & Hudgens, M. G. (2020). Group Testing for Severe Acute Respiratory Syndrome-Coronavirus 2 to Enable Rapid Scale-up of Testing and Real-Time Surveillance of Incidence. *The Journal of infectious diseases*, *222*(6), 903–909. https://doi.org/10.1093/infdis/jiaa378
154. Priye, A., Bird, S. W., Light, Y. K., Ball, C. S., Negrete, O. A., & Meagher, R. J. (2017). A smartphone-based diagnostic platform for rapid detection of Zika, chikungunya, and dengue viruses. *Scientific reports*, *7*, 44778. https://doi.org/10.1038/srep44778
155. Ptasinska, A., Whalley, C., Bosworth, A., Poxon, C., Bryer, C., Machin, N., Grippon, S., Wise, E., Armson, B., Howson, E., Goring, A., Snell, G., Forster, J., Mattocks, C., Frampton, S., Anderson, R., Cleary, D., Parker, J., Boukas, K., Graham, N., Cellura, D., Garratt, E., Skilton, R., Sheldon, H., Collins, A., Ahmad, N., Friar, S., Williams, T., Godfrey, K., Deans, Z., Douglas, A., Hill, S., Kidd, M., Porter, D., Kidd, S., Cortes, N., Fowler, V., Williams, T., Richter, A., & Beggs, A. (2020). Diagnostic accuracy of Loop mediated isothermal amplification coupled to Nanopore sequencing (LamPORE) for the detection of SARS-CoV-2 infection at scale in symptomatic and asymptomatic populations. *medRxiv*.
156. Qin, Z., Peng, R., Baravik, I. K., & Liu, X. (2020). Fighting COVID-19: Integrated Micro- and Nanosystems for Viral Infection Diagnostics. *Matter*, *3*(3), 628–651. https://doi.org/10.1016/j.matt.2020.06.015
157. Quyen, T. L., Nordentoft, S., Vinayaka, A. C., Ngo, T. A., Engelsmenn, P., Sun, Y., Madsen, M., Bang, D. D., & Wolff, A. (2019). A Sensitive, Specific and Simple Loop Mediated Isothermal Amplification Method for Rapid Detection of *Campylobacter* spp. in Broiler Production. *Frontiers in microbiology*, *10*, 2443. https://doi.org/10.3389/fmicb.2019.02443
158. Rabe, B. A., & Cepko, C. (2020). SARS-CoV-2 detection using isothermal amplification and a rapid, inexpensive protocol for sample inactivation and purification. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(39), 24450–24458. https://doi.org/10.1073/pnas.2011221117
159. Ranoa, D., Holland, R., Alnaji, F., Green, K., Wang, L., Brooke, C., Burke, M., Fan, T., & Hergenrother, P. (2020). Saliva-Based Molecular Testing for SARS-CoV-2 that Bypasses RNA Extraction. *bioRxiv*.
160. Reboud, J., Xu, G., Garrett, A., Adriko, M., Yang, Z., Tukahebwa, E. M., Rowell, C., & Cooper, J. M. (2019). Paper-based microfluidics for DNA diagnostics of malaria in low resource underserved rural communities. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(11), 4834–4842. https://doi.org/10.1073/pnas.1812296116
161. Rendeiro AF, Ravichandran H, Bram Y, Chandar V, Kim J, Meydan C, Park J, Foox J, Hether T, Warren S, Kim Y, Reeves J, Salvatore S, Mason CE, Swanson EC, Borczuk AC, Elemento O, Schwartz RE. The spatial landscape of lung pathology during COVID-19 progression. *Nature*. 2021 Mar 29.
162. Rei CK, Li C, Bertrand D, Ng AHQ, Kwah JS, Low HM, Tong C, Natrajan M, Zhang MH, Xu L, Ko KK, Ho EXP, Av-Shalom TV, Teo JWP, Khor CC, MetaSUB Consortium, Chen SL, Mason CE, Ng OT, Marimuthu K, Ang B, Nagarajan N. Cartography of opportunistic pathogens and antibiotic resistance genes in a tertiary hospital environment. *Nature Medicine*. 2020. Jun 8. 10:2, 42-48.
163. Rodriguez-Manzano, J., Malpartida-Cardenas, K., Moser, N., Pennisi, I., Cavuto, M., Miglietta, L., Moniri, A., Penn, R., Satta, G., Randell, P., Davies, F., Bolt, F., Barclay, W., Holmes, A., & Georgiou, P. (2021). Handheld Point-of-

Care System for Rapid Detection of SARS-CoV-2 Extracted RNA in under 20 min. *ACS central science*, *7*(2), 307–317. https://doi.org/10.1021/acscentsci.0c01288

164. Rohaim, M. A., Clayton, E., Sahin, I., Vilela, J., Khalifa, M. E., Al-Natour, M. Q., Bayoumi, M., Poirier, A. C., Branavan, M., Tharmakulasingam, M., Chaudhry, N. S., Sodi, R., Brown, A., Burkhart, P., Hacking, W., Botham, J., Boyce, J., Wilkinson, H., Williams, C., Whittingham-Dowd, J., … Munir, M. (2020). Artificial Intelligence-Assisted Loop Mediated Isothermal Amplification (AI-LAMP) for Rapid Detection of SARS-CoV-2. *Viruses*, *12*(9), 972. https://doi.org/10.3390/v12090972
165. Rolando, J. C., Jue, E., Barlow, J. T., & Ismagilov, R. F. (2020). Real-time kinetics and high-resolution melt curves in single-molecule digital LAMP to differentiate and study specific and non-specific amplification. *Nucleic acids research*, *48*(7), e42. https://doi.org/10.1093/nar/gkaa099
166. Rolando, J. C., Jue, E., Schoepp, N. G., & Ismagilov, R. F. (2019). Real-Time, Digital LAMP with Commercial Microfluidic Chips Reveals the Interplay of Efficiency, Speed, and Background Amplification as a Function of Reaction Temperature and Time. *Analytical chemistry*, *91*(1), 1034–1042. https://doi.org/10.1021/acs.analchem.8b04324
167. Roy, S., Mohd-Naim, N. F., Safavieh, M., & Ahmed, M. U. (2017). Colorimetric Nucleic Acid Detection on Paper Microchip Using Loop Mediated Isothermal Amplification and Crystal Violet Dye. *ACS sensors*, *2*(11), 1713–1720. https://doi.org/10.1021/acssensors.7b00671
168. Safavieh, M., Kaul, V., Khetani, S., Singh, A., Dhingra, K., Kanakasabapathy, M. K., Draz, M. S., Memic, A., Kuritzkes, D. R., & Shafiee, H. (2017). Paper microchip with a graphene-modified silver nano-composite electrode for electrical sensing of microbial pathogens. *Nanoscale*, *9*(5), 1852–1861. https://doi.org/10.1039/c6nr06417e
169. Saletore Y, Meyer K, Korlach J, Vilfan I, Jaffrey S, Mason CE. "The Birth of the Epitranscriptome: Deciphering the Function of RNA Modifications." *Genome Biology*. 2012 Oct 31;13(10):175.
170. Sapoval, N., Mahmoud, M., Jochum, M. D., Liu, Y., Elworth, R., Wang, Q., Albin, D., Ogilvie, H., Lee, M. D., Villapol, S., Hernandez, K. M., Berry, I. M., Foox, J., Beheshti, A., Ternus, K., Aagaard, K. M., Posada, D., Mason, C. E., Sedlazeck, F., & Treangen, T. J. (2020). Hidden genomic diversity of SARS-CoV-2: implications for qRT-PCR diagnostics and transmission. *bioRxiv : the preprint server for biology*, 2020.07.02.184481. https://doi.org/10.1101/2020.07.02.184481
171. Schellenberg, J. J., Ormond, M., & Keynan, Y. (2021). Extraction-free RT-LAMP to detect SARS-CoV-2 is less sensitive but highly specific compared to standard RT-PCR in 101 samples. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*, *136*, 104764. https://doi.org/10.1016/j.jcv.2021.104764
172. Schmid-Burgk, J., Schmithausen, R., Li, D., Hollstein, R., Ben-Shmuel, A., Israeli, O., Weiss, S., Paran, N., Wilbring, G., Liebing, J., Feldman, D., S\labicki, M., Lippke, B., Sib, E., Borrajo, J., Strecker, J., Reinhardt, J., Hoffmann, P., Cleary, B., Hölzel, M., Nöthen, M., Exner, M., Ludwig, K., Regev, A., & Zhang, F. (2020). LAMP-Seq: Population-Scale COVID-19 Diagnostics Using Combinatorial Barcoding. *bioRxiv*.
173. Schönbrunner, N. J., Fiss, E. H., Budker, O., Stoffel, S., Sigua, C. L., Gelfand, D. H., & Myers, T. W. (2006). Chimeric thermostable DNA polymerases with reverse transcriptase and attenuated 3'-5' exonuclease activity. *Biochemistry*, *45*(42), 12786–12795. https://doi.org/10.1021/bi0609117
174. Schuler, F., Siber, C., Hin, S., Wadle, S., Paust, N., Zengerle, R., & Stetten, F. (2016). Digital droplet LAMP as a microfluidic app on standard laboratory devices. *Anal. Methods*, *8*, *2750-2755*.
175. Seok, Y., Joung, H. A., Byun, J. Y., Jeon, H. S., Shin, S. J., Kim, S., Shin, Y. B., Han, H. S., & Kim, M. G. (2017). A Paper-Based Device for Performing Loop-Mediated Isothermal Amplification with Real-Time Simultaneous Detection of Multiple DNA Targets. *Theranostics*, *7*(8), 2220–2230. https://doi.org/10.7150/thno.18675
176. Seok, Y., Joung, H. A., Byun, J. Y., Jeon, H. S., Shin, S. J., Kim, S., Shin, Y. B., Han, H. S., & Kim, M. G. (2017). A Paper-Based Device for Performing Loop-Mediated Isothermal Amplification with Real-Time Simultaneous Detection of Multiple DNA Targets. *Theranostics*, *7*(8), 2220–2230. https://doi.org/10.7150/thno.18675
177. SEQC/MAQC-III Consortium. "A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequence Quality Control consortium." *Nature Biotechnology*. 2014 Sep;32(9):903-14.
178. Seyrig, G., Stedtfeld, R. D., Tourlousse, D. M., Ahmad, F., Towery, K., Cupples, A. M., Tiedje, J. M., & Hashsham, S. A. (2015). Selection of fluorescent DNA dyes for real-time LAMP with portable and simple optics. *Journal of microbiological methods*, *119*, 223–227. https://doi.org/10.1016/j.mimet.2015.11.004
179. Sherrill-Mix, S., Duyne, G., & Bushman, F. (2021). Molecular beacons allow specific RT-LAMP detection of B.1.1.7 variant SARS-CoV-2. *medRxiv*.

180. Sherrill-Mix, S., Hwang, Y., Roche, A., Glascock, A., Weiss, S., Li, Y., Haddad, L., Deraska, P., Monahan, C., Kromer, A., Graham-Wooten, J., Taylor, L., Abella, B., Ganguly, A., Collman, R., Van Duyne, G., & Bushman, F. (2021). LAMP-BEAC: Detection of SARS-CoV-2 RNA Using RT-LAMP and Molecular Beacons. *medRxiv*.
181. Shirato, K., Yano, T., Senba, S., Akachi, S., Kobayashi, T., Nishinaka, T., Notomi, T., & Matsuyama, S. (2014). Detection of Middle East respiratory syndrome coronavirus using reverse transcription loop-mediated isothermal amplification (RT-LAMP). *Virology journal*, *11*, 139. https://doi.org/10.1186/1743-422X-11-139
182. Silva, A., Lima-Neto, L. G., Azevedo, C., Costa, L., Bragança, M., Barros Filho, A., Wittlin, B. B., Souza, B. F., Oliveira, B., Carvalho, C. A., Thomaz, E., Simões-Neto, E. A., Leite Júnior, J. F., Cosme, L., Campos, M., Queiroz, R., Costa, S. S., Carvalho, V. A., Simões, V., Alves, M., … Santos, A. (2020). Population-based seroprevalence of SARS-CoV-2 and the herd immunity threshold in Maranhão. *Revista de saude publica*, *54*, 131. https://doi.org/10.11606/s1518-8787.2020054003278
183. Singleton, J., Osborn, J. L., Lillis, L., Hawkins, K., Guelig, D., Price, W., Johns, R., Ebels, K., Boyle, D., Weigl, B., & LaBarre, P. (2014). Electricity-free amplification and detection for molecular point-of-care diagnosis of HIV-1. *PloS one*, *9*(11), e113693. https://doi.org/10.1371/journal.pone.0113693
184. Snodgrass, R., Gardner, A., Jiang, L., Fu, C., Cesarman, E., & Erickson, D. (2016). KS-Detect - Validation of Solar Thermal PCR for the Diagnosis of Kaposi's Sarcoma Using Pseudo-Biopsy Samples. *PloS one*, *11*(1), e0147636. https://doi.org/10.1371/journal.pone.0147636
185. Snodgrass, R., Gardner, A., Semeere, A., Kopparthy, V. L., Duru, J., Maurer, T., Martin, J., Cesarman, E., & Erickson, D. (2018). A portable device for nucleic acid quantification powered by sunlight, a flame or electricity. *Nature biomedical engineering*, *2*(9), 657–665. https://doi.org/10.1038/s41551-018-0286-y
186. Soares, R., Akhtar, A., Pinto, I., Lapins, N., Barrett, D., Sandh, G., Yin, X., Pelechano, V., & Russom, A. (2020). Point-of-care detection of SARS-CoV-2 in nasopharyngeal swab samples using an integrated smartphone-based centrifugal microfluidic platform. *medRxiv*.
187. Song, J., Mauk, M. G., Hackett, B. A., Cherry, S., Bau, H. H., & Liu, C. (2016). Instrument-Free Point-of-Care Molecular Detection of Zika Virus. *Analytical chemistry*, *88*(14), 7289–7294. https://doi.org/10.1021/acs.analchem.6b01632
188. Stedtfeld, R. D., Tourlousse, D. M., Seyrig, G., Stedtfeld, T. M., Kronlein, M., Price, S., Ahmad, F., Gulari, E., Tiedje, J. M., & Hashsham, S. A. (2012). Gene-Z: a device for point of care genetic testing using a smartphone. *Lab on a chip*, *12*(8), 1454–1462. https://doi.org/10.1039/c2lc21226a
189. Sun, F., Ganguli, A., Nguyen, J., Brisbin, R., Shanmugam, K., Hirschberg, D., Wheeler, M., Bashir, R., Nash, D., & Cunningham, B. (2020). Smartphone-based multiplex 30-minute nucleic acid test of live virus from nasal swab extract. *Lab Chip*, *20*, *1621-1627*.
190. Takayama, I., Semba, S., Yokono, K., Saito, S., Nakauchi, M., Kubo, H., Kaida, A., Shiomi, M., Terada, A., Murakami, K., Kaji, K., Kiya, K., Sawada, Y., Oba, K., Asai, S., Yonekawa, T., Watanabe, H., Segawa, Y., Notomi, T., & Kageyama, T. (2020). Clinical evaluation of fully automated molecular diagnostic system "Simprova" for influenza virus, respiratory syncytial virus, and human metapneumovirus. *Scientific reports*, *10*(1), 13496. https://doi.org/10.1038/s41598-020-70090-2
191. Tanner, N. A., Zhang, Y., & Evans, T. C., Jr (2012). Simultaneous multiple target detection in real-time loop-mediated isothermal amplification. *BioTechniques*, *53*(2), 81–89. https://doi.org/10.2144/0000113902
192. Tighe S, Afshinnekoo E, Green SJ, McGrath K, Alexander N, McIntyre A, Baldwin DA, Joye S, Johnson SS, Bivens N, Carmical R, et al. "Genomic Methods and Microbiological Technologies for Profiling Novel and Extreme Environments for The Extreme Microbiome Project (XMP)." *Journal of Biomolecular Techniques.* 2017 Apr;28(1):31-39.
193. To, K. K., Tsang, O. T., Yip, C. C., Chan, K. H., Wu, T. C., Chan, J. M., Leung, W. S., Chik, T. S., Choi, C. Y., Kandamby, D. H., Lung, D. C., Tam, A. R., Poon, R. W., Fung, A. Y., Hung, I. F., Cheng, V. C., Chan, J. F., & Yuen, K. Y. (2020). Consistent Detection of 2019 Novel Coronavirus in Saliva. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, *71*(15), 841–843. https://doi.org/10.1093/cid/ciaa149
194. Torres, C., Vitalis, E. A., Baker, B. R., Gardner, S. N., Torres, M. W., & Dzenitis, J. M. (2011). LAVA: an open-source approach to designing LAMP (loop-mediated isothermal amplification) DNA signatures. *BMC bioinformatics*, *12*, 240. https://doi.org/10.1186/1471-2105-12-240
195. Tran, D., Cuong, H., Tran, H., Le, U., Do, H., Bui, L., Hai, N., Linh, H., Thao, N., Anh, N., Hieu, N., Thang, C., Vu, V., & Phung, H. (2021). A comparative study of isothermal nucleic acid amplification methods for SARS-CoV-2 detection at point-of-care. *bioRxiv*.

196. Tyagi, S., & Kramer, F. R. (1996). Molecular beacons: probes that fluoresce upon hybridization. *Nature biotechnology*, *14*(3), 303–308. https://doi.org/10.1038/nbt0396-303
197. Urdea, M., Penny, L. A., Olmsted, S. S., Giovanni, M. Y., Kaspar, P., Shepherd, A., Wilson, P., Dahl, C. A., Buchsbaum, S., Moeller, G., & Hay Burgess, D. C. (2006). Requirements for high impact diagnostics in the developing world. *Nature*, *444 Suppl 1*, 73–79. https://doi.org/10.1038/nature05448
198. van Dongen, J. E., Berendsen, J., Steenbergen, R., Wolthuis, R., Eijkel, J., & Segerink, L. I. (2020). Point-of-care CRISPR/Cas nucleic acid detection: Recent advances, challenges and opportunities. *Biosensors & bioelectronics*, *166*, 112445. https://doi.org/10.1016/j.bios.2020.112445
199. Varsha, V., Aishwarya, S., Murchana, S., Naveen, G., Ramya, M., & Rathinasabapathi, P. (2020). Correction pen based paper fluidic device for the detection of multiple gene targets of Leptospira using Loop Mediated Isothermal Amplification. *Journal of microbiological methods*, *174*, 105962. https://doi.org/10.1016/j.mimet.2020.105962
200. Velders, A. H., Schoen, C., & Saggiomo, V. (2018). Loop-mediated isothermal amplification (LAMP) shield for Arduino DNA detection. *BMC research notes*, *11*(1), 93. https://doi.org/10.1186/s13104-018-3197-9
201. Vogels, C., Watkins, A. E., Harden, C. A., Brackney, D. E., Shafer, J., Wang, J., Caraballo, C., Kalinich, C. C., Ott, I. M., Fauver, J. R., Kudo, E., Lu, P., Venkataraman, A., Tokuyama, M., Moore, A. J., Muenker, M. C., Casanovas-Massana, A., Fournier, J., Bermejo, S., Campbell, M., … Grubaugh, N. D. (2021). SalivaDirect: A simplified and flexible platform to enhance SARS-CoV-2 testing capacity. *Med (New York, N.Y.)*, *2*(3), 263–280.e6. https://doi.org/10.1016/j.medj.2020.12.010.
202. Vogels CBF, Breban MI, Ott IM, Alpert T, Petrone ME, Watkins AE, Kalinich CC, Earnest R, Rothman JE, Goes de Jesus J, Morales Claro I, Magalhães Ferreira G, Crispim MAE; Brazil-UK CADDE Genomic Network, Singh L, Tegally H, Anyaneji UJ; Network for Genomic Surveillance in South Africa, Hodcroft EB, Mason CE, Khullar G, Metti J, Dudley JT, MacKay MJ, Nash M, Wang J, Liu C, Hui P, Murphy S, Neal C, Laszlo E, Landry ML, Muyombwe A, Downing R, Razeq J, de Oliveira T, Faria NR, Sabino EC, Neher RA, Fauver JR, Grubaugh ND. Multiplex qPCR discriminates variants of concern to enhance global surveillance of SARS-CoV-2. *PLoS Biology*. 2021 May 7;19(5):e3001236.
203. Wei, S., Kohl, E., Djandji, A., Morgan, S., Whittier, S., Mansukhani, M., Hod, E., D'Alton, M., Suh, Y., & Williams, Z. (2020). Direct diagnostic testing of SARS-CoV-2 without the need for prior RNA extraction. *medRxiv : the preprint server for health sciences*, 2020.05.28.20115220. https://doi.org/10.1101/2020.05.28.20115220
204. Westhaus, S., Widera, M., Rabenau, H., Hoehl, S., Bojkova, D., Cinatl, J., & Ciesek, S. (2020). Evaluation of stability and inactivation methods of SARS-CoV-2 in context of laboratory settings. *bioRxiv*.
205. Wölfel, R., Corman, V. M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M. A., Niemeyer, D., Jones, T. C., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Brünink, S., Schneider, J., Ehmann, R., Zwirglmaier, K., Drosten, C., & Wendtner, C. (2020). Virological assessment of hospitalized patients with COVID-2019. *Nature*, *581*(7809), 465–469. https://doi.org/10.1038/s41586-020-2196-x
206. Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., Song, Z. G., Hu, Y., Tao, Z. W., Tian, J. H., Pei, Y. Y., Yuan, M. L., Zhang, Y. L., Dai, F. H., Liu, Y., Wang, Q. M., Zheng, J. J., Xu, L., Holmes, E. C., & Zhang, Y. Z. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, *579*(7798), 265–269. https://doi.org/10.1038/s41586-020-2008-3
207. Xu, J., Wang, J., Zhong, Z., Su, X., Yang, K., Chen, Z., Zhang, D., Li, T., Wang, Y., Zhang, S., Ge, S., Zhang, J., & Xia, N. (2020). Room-temperature-storable PCR mixes for SARS-CoV-2 detection. *Clinical biochemistry*, *84*, 73–78. https://doi.org/10.1016/j.clinbiochem.2020.06.013
208. Yager, P., Domingo, G. J., & Gerdes, J. (2008). Point-of-care diagnostics for global health. *Annual review of biomedical engineering*, *10*, 107–144. https://doi.org/10.1146/annurev.bioeng.10.061807.160524
209. Yan, C., Cui, J., Huang, L., Du, B., Chen, L., Xue, G., Li, S., Zhang, W., Zhao, L., Sun, Y., Yao, H., Li, N., Zhao, H., Feng, Y., Liu, S., Zhang, Q., Liu, D., & Yuan, J. (2020). Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, *26*(6), 773–779. https://doi.org/10.1016/j.cmi.2020.04.001
210. Yang, Q., Meyerson, N., Clark, S., Paige, C., Fattor, W., Gilchrist, A., Barbachano-Guerrero, A., Healy, B., Worden-Sapper, E., Wu, S., Muhlrad, D., Decker, C., Saldi, T., Lasda, E., Gonzales, P., Fink, M., Tat, K., Hager, C., Davis, J., Ozeroff, C., Brisson, G., McQueen, M., Leinwand, L., Parker, R., & Sawyer, S. (2021). Saliva TwoStep for rapid detection of asymptomatic SARS-CoV-2 carriers. *medRxiv*.

211. Yaren, O., McCarter, J., Phadke, N., Bradley, K. M., Overton, B., Yang, Z., Ranade, S., Patil, K., Bangale, R., & Benner, S. A. (2021). Ultra-rapid detection of SARS-CoV-2 in public workspace environments. *PloS one*, *16*(2), e0240524. https://doi.org/10.1371/journal.pone.0240524
212. Yasukawa, K., Yanagihara, I., & Fujiwara, S. (2020). Alteration of enzymes and their application to nucleic acid amplification (Review). *International journal of molecular medicine*, *46*(5), 1633–1643. https://doi.org/10.3892/ijmm.2020.4726
213. Yelagandula, R., Bykov, A., Vogt, A., Heinen, R., \"Ozkan, E., Strobl, M., Baar, J., Uzunova, K., Hajdusits, B., Kordic, D., Suljic, E., Kurtovic-Kozaric, A., Izetbegovic, S., Schaefer, J., Hufnagl, P., Zoufaly, A., Seitz, T., , Födinger, M., Allerberger, F., Stark, A., Cochella, L., & Elling, U. (2020). SARSeq, a robust and highly multiplexed NGS assay for parallel detection of SARS-CoV2 and other respiratory infections. *medRxiv*.
214. Yoo, W., Han, H., Kim, J., Kang, K., Jeon, H.S., Moon, J.Y., & Park, H. (2020). Development of a tablet PC-based portable device for colorimetric determination of assays including COVID-19 and other pathogenic microorganisms. *RSC Adv., 10, 32946-32952.*
215. Yoshikawa, R., Abe, H., Igasaki, Y., Negishi, S., Goto, H., & Yasuda, J. (2020). Development and evaluation of a rapid and simple diagnostic assay for COVID-19 based on loop-mediated isothermal amplification. *PLoS neglected tropical diseases*, *14*(11), e0008855. https://doi.org/10.1371/journal.pntd.0008855
216. Yu, C. Y., Chan, K. G., Yean, C. Y., & Ang, G. Y. (2021). Nucleic Acid-Based Diagnostic Tests for the Detection SARS-CoV-2: An Update. *Diagnostics (Basel, Switzerland)*, *11*(1), 53. https://doi.org/10.3390/diagnostics11010053
217. Yu, L., Wu, S., Hao, X., Dong, X., Mao, L., Pelechano, V., Chen, W. H., & Yin, X. (2020). Rapid Detection of COVID-19 Coronavirus Using a Reverse Transcriptional Loop-Mediated Isothermal Amplification (RT-LAMP) Diagnostic Platform. *Clinical chemistry*, *66*(7), 975–977. https://doi.org/10.1093/clinchem/hvaa102
218. Yuan, H., Chao, Y., & Shum, H. C. (2020). Droplet and Microchamber-Based Digital Loop-Mediated Isothermal Amplification (dLAMP). *Small (Weinheim an der Bergstrasse, Germany)*, *16*(9), e1904469. https://doi.org/10.1002/smll.201904469
219. Zhang, C., Zheng, T., Wang, H., Chen, W., Huang, X., Liang, J., Qiu, L., Han, D., & Tan, W. (2021). Rapid One-Pot Detection of SARS-CoV-2 Based on a Lateral Flow Assay in Clinical Samples. *Analytical chemistry*, *93*(7), 3325–3330. https://doi.org/10.1021/acs.analchem.0c05059
220. Zhang, H., Xu, Y., Fohlerova, Z., Chang, H., Iliescu, C., & Neuzil, P. (2019). LAMP-on-a-chip: Revising microfluidic platforms for loop-mediated DNA amplification. *Trends in analytical chemistry : TRAC*, *113*, 44–53. https://doi.org/10.1016/j.trac.2019.01.015
221. Zhang, X. O., Wang, H. B., Zhang, Y., Lu, X., Chen, L. L., & Yang, L. (2014). Complementary sequence-mediated exon circularization. *Cell*, *159*(1), 134–147. https://doi.org/10.1016/j.cell.2014.09.001
222. Zhang, Y., Odiwuor, N., Xiong, J., Sun, L., Nyaruaba, R., Wei, H., & Tanner, N. (2020). Rapid Molecular Detection of SARS-CoV-2 (COVID-19) Virus RNA Using Colorimetric LAMP. *medRxiv*.
223. Zhang, Y., Ren, G., Buss, J., Barry, A. J., Patton, G. C., & Tanner, N. A. (2020). Enhancing colorimetric loop-mediated isothermal amplification speed and sensitivity with guanidine chloride. *BioTechniques*, *69*(3), 178–185. https://doi.org/10.2144/btn-2020-0078
224. Zhang, Y., Ren, G., Buss, J., Barry, A. J., Patton, G. C., & Tanner, N. A. (2020). Enhancing colorimetric loop-mediated isothermal amplification speed and sensitivity with guanidine chloride. *BioTechniques*, *69*(3), 178–185. https://doi.org/10.2144/btn-2020-0078
225. Zhu, J., Guo, J., Xu, Y., & Chen, X. (2020). Viral dynamics of SARS-CoV-2 in saliva from infected patients. *The Journal of infection*, *81*(3), e48–e50. https://doi.org/10.1016/j.jinf.2020.06.059