RT-LAMP AND CRISPR APPLICATIONS IN COVID-19 DETECTION

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ABSTRACT

SARS-CoV-2, more commonly known as COVID-19, is a novel coronavirus that has spread on a global scale since its emergence in late 2019. The ongoing pandemic fueled researchers to study new COVID-19 detection methods that are more efficient and accurate alongside new mutations that are coming to light. COVID-19 testing has been heavily dominated by the utilization of polymerase chain reactions (PCR). However, PCR testing often takes an extended period of time before individuals receive their results and requires the precision of skilled personnel to handle scientific equipment and conduct readings. PCR testing requires a large number of resources to conduct, making it difficult for individuals within the general community to conveniently and quickly get tested in the case of close exposure. Thus, researchers around the world have been looking for alternative methods of COVID-19 detection that not only are fast and convenient for test-takers but also maintain the same degree of accuracy. Researchers studying genome editing and subsequent CRISPR-Cas systems have found applications to aid in COVID-19 detection. The CRISPR-Cas system coupled with pre-existing Reverse Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP) methods was discovered to be a viable means of detecting COVID-19 in humans. The combined RT-LAMP and CRISPR-Cas system form of COVID-19 testing proves useful in practice because it requires less machinery, fewer trained individuals to monitor the reactions, and is an overall simpler procedure to perform in larger numbers; subsequently making this new form of COVID-19 detection more viable on a worldwide level. This discussion intends to explore the different experiments on utilizing this new and effective COVID-19 detection method. The drawbacks and limitations of the experiment will also be outlined, as well as implementing this detection method in hopes to revolutionize the future of worldwide disease detection.

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INTRODUCTION

Cases of a novel coronavirus began to emerge in late 2019 in Wuhan, the Chinese capital of Hubei Province home to over 11 million people. Cough, fever, and runny nose were some of the common symptoms of this newly surfaced viral infection which also overlapped with other common illnesses such as seasonal influenza. However, serious cases of this viral infection resulted in permanent medical ailments and death. Wuhanese citizens were extremely susceptible to the novel coronavirus COVID-19 (SARS-CoV-2) due to various factors including the area's dense population, commonality of infection symptoms, and lack of scientific knowledge on how to prevent the spread of this novel virus. These combined aspects brought forth a large wave of COVID-19 cases in Wuhan and subsequently other international countries due to travel (Worobey, 2021). Nations mobilized as quickly as they could to reduce the spread of the virus through the implementation of medical-grade face masks, social distancing protocols, halting international travel, locking down traveling outside cities, and diverting resources to scientists to research and study the virus to make an effective vaccine. In the following two years, not only did COVID-19 cases continue to rise and surpass millions of cases worldwide, various vaccinations were dispersed to reduce the fatal effects of COVID-19 infection.

The prevalence of COVID-19 cases also brought forth varied methods to effectively test asymptomatic and symptomatic individuals for viral infection. Presently, polymerase chain reaction (PCR) testing is the most common form of COVID-19 testing; it relies upon the amplification of a target sequence of the gene to a high degree to identify whether or not the genome has been infected by the virus. This mode of testing has been proven to have a high accuracy rate in detecting COVID-19 infection, but is relatively time-consuming, taking up to 5

days or more for results in some areas, and resource-intensive as it relies on results being interpreted by various laboratory mechanisms and highly trained scientists. Despite being an effective means of testing, it is not practical for individuals to get quickly tested at convenient locations. Thus, finding a new means of accurately and conveniently testing people for COVID-19 with as few resources as possible has become a priority task for researchers worldwide.

Researchers studying gene editing, specifically CRISPR-Cas systems, began looking into methods to apply the technology to COVID-19 testing. Clustered Regularly Interspaced Short Palindromic Repeats, also known as CRISPR, refers to an intricate system that allows genomes to be specifically modified. This advancement is still being studied and understood, but it is becoming increasingly crucial to modern medical advancements. CRISPR consists of two key components, a guide RNA (gRNA) that allows the CRISPR system to be led to the DNA segment of interest and also the CRISPR-Cas endonuclease complex which cleaves the DNA segment of interest (Vidyasagar, 2021). The CRISPR method is based on a natural method bacteria use in order to fight against bacteriophages that invade and infect them. It works in bacteria by having a gRNA that searches for the viral DNA in the host. Once it finds it, the CRISPR Cas-9 system attaches itself to the sequence of interest and cuts it, rendering it inactive and disallowing its transcription in a cell (therefore forbidding the viral DNA from replicating and damaging the cell). This method used by bacteria became useful because it allowed new ideas to emerge rendering its usefulness to humans. CRISPR is able to be coupled with different CRISPR-Cas enzymes which allow it to participate in different functions.

Utilization of this CRISPR-Cas system coupled with continued research in the Reverse Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP) methodology showcased its

usefulness as a possible COVID-19 detection method. RT-LAMP is a single-step process that allows for nucleic acids to be identified and amplificated isothermally via loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) (Ryding, 2021). The viral detection mechanism utilizes both RT-LAMP and RT-RPA assays coupled with CRISPR enzymes (Cas9, Cas12, and Cas13) to aid in increasing the sensitivity of the detection of nucleic acids and viruses including SARS-CoV-2. The CRISPR Cas12 and Cas13 enzymes specifically interact with their respective single-guide RNA (sgRNA) strands and scan the complementary DNA/RNA template to create a corresponding sequence. When this sequence is recognized, the enzymes are signaled to cleave off the cis-nucleic acid, which is the viral component needed to be identified. The enzyme also performs trans-cleavage action by cleaving associated single-stranded (ss) DNA/RNA molecules that are involved in the reaction. The ability of the Cas12 and Cas13 enzymes to cleave nucleic acids allows for effective viral material detection. The CRISPR-Cas12/13 system coupled with RPA is utilized for virus detection via SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) and ultimately does not require the extra step to extract and purify the nucleic acids for detection (Kelner et. al, 2019).

DATA AND METHODS

Researchers designed a “one-pot” CRISPR-Cas 12 RT-Lamp system. To decide which cRNA would be used for the Cas12a system, they tested 7 different cRNA sequences by incubating them with synthetic target DNA, Cas12a, the ssDNA reporters (Fig. 1). They found that cRNA targeting the S gene of the COVID-19 genome produced the strongest fluorescence signals, and so this was used in the one-pot CRISPR system.

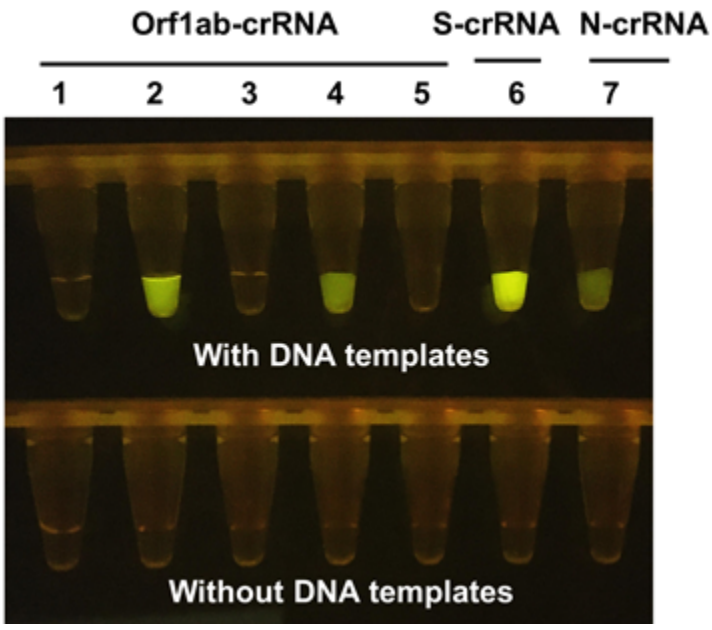

Fig. 1 Fluorescence of incubation of different cRNA sequences with and without the target DNA (Wang et. al, 2020)

The researchers also attempted to optimize the RT-LAMP mixture to Cas12a mixture ratio. They did this by first allowing the RT-LAMP mixture to run at 65°C for 40 minutes with mineral oil on top of the RT-Lamp mixture to reduce heat transfer and to keep the Cas12a enzyme active. Each RT-LAMP mixture had either a fixed concentration or a fixed number of copies of the target RNA, and control samples had no template added. They tested adding different volumes of the RT-LAMP mixture to 20 µL of Cas12a mixture and letting the reaction run for 10 minutes at 37°C. They decided to continue further optimizations with 20 µL of Cas12a mixture and 40 µL RT-LAMP mixture after finding that 5 to 40 µL "generated significant fluorescence" (Fig 2).

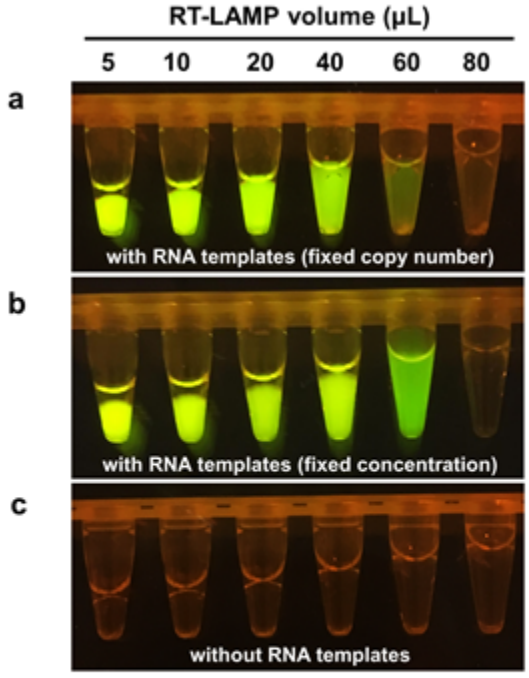

Fig 2. Row A, B, and C depict the fluorescence of different ratios of the RT-LAMP mixture to the Cas12 enzyme mixture utilizing a fixed number of target RNA, a fixed concentration of target RNA, and the absence of RNA templates respectively.

Scientists then tested this one-pot method on "three SARS-like coronaviruses (SARS-CoV-2 (Genebank: NC_045512.2), bat SARS-like coronavirus (bat-SL-CoVZC45, Genebank: MG772933.1) and SARS-CoV (Genebank: NC_004718.3)) and one human coronaviruses (HKU1 (Genebank: NC_006577.) and found that fluorescence was only reported from the SARS-CoV-2 (COVID-19) sample.

The researchers then tested to optimize cleavage reaction time. They tested the one-pot process at different times, using heat inactivation to stop the Cas12a cleavage process at the desired times. They found that as the time increased, fluorescence strength increased up until the 5-minute mark. Distilled water instead of RNA template and uninfected patient RNA samples

were used as blank and negative controls, respectively and it was found that there was no fluorescence in negative control up to the ten-minute mark. Thus, the 5-minute cleavage reaction time was chosen for the one-pot procedure (Fig 3).

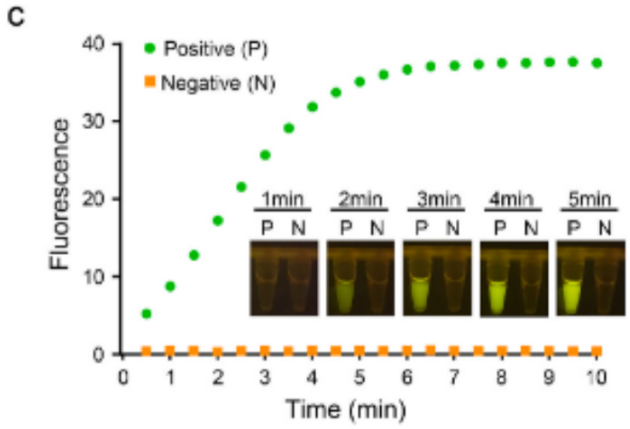

Fig 3. Graph of fluorescence of cleavage reaction results against time to determine optimal reaction time.

The researchers also tested the sensitivity of the one-pot procedure by running this procedure with “eight 10-fold serially diluted RNA templates” (Wang et. al, 2020). In comparison to PCR and RT-LAMP without CRISPR, it was found that one pot RT-LAMP with CRISPR has comparable sensitivity to PCR and 10 times greater sensitivity than RT-LAMP without CRISPR. The detection limit of the one-pot procedure was 5 copies. For the one-pot procedure, by stopping the RT-LAMP reaction at different times and then running the cleavage reaction, it was found that the “amplicons accumulated by RT-LAMP within 20 min were below the fluorescence threshold” by themselves, they were detectable with CRISPR Cas12 (Wang et. al, 2020).

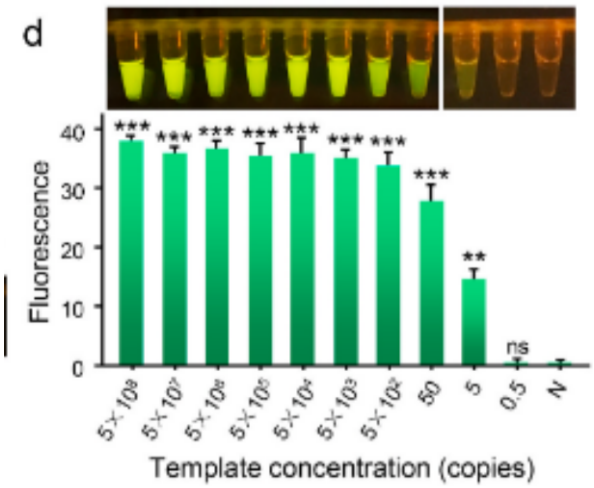

Fig 4. Graph of fluorescence against template concentration.

To test accuracy, the one-pot procedure and PCR were tested on 26 samples verified to be Sars-CoV-2 positive and 24 samples that were not infected. For both PCR and One-Pot procedures, all positive samples yielded positive results and all uninfected samples yielded negative results.

To conclude, they created 40 µL RT-LAMP reaction mixture which included RNA (either template RNA produced from in vitro transcription or RNA from clinical samples) in a tube and covered this mixture in 25 µL of mineral oil. They also added a mixture of "0.4 µM of EnGenLba Cas12a, 2 × NEB buffer 2.1, 1.2 µM of crRNA, 2 µM of ssDNA reporter, RNase inhibitor 8 U" to the lid of the tube to serve as the Cas12a system (Wang et. al, 2020). This allows amplification of the RNA sample to occur, and then recognition of the target sequence using CRISPR could be started by shaking the tube to mix in the Cas12a system. The researchers let amplification occur for 40 minutes before shaking the tube. Cleavage via CRISPR occurred at 37°C for 5 minutes and fluorescence was observed using a blue light illuminator.

Researchers also developed methods incorporating RT-LAMP and CRISPR as a DETECTR ("SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter") system using lateral flow assays. In "CRISPR–Cas12-based detection of SARS-CoV-2", the researchers developed a procedure involving RT-LAMP, CRISPR, lateral flow assays, and specifically designed primers for the RT-LAMP process. The researchers designed primers "targeting the E (envelope) and N (nucleoprotein) genes of SARS-CoV-2" (Broughton et. al, 2020) using PrimerExplorer v.5, and also designed guide RNAs that detect strictly the N gene of SARS-CoV-2 and "three SARS-like coronaviruses (SARS-CoV-2 (accession NC_045512), bat SARS-like coronavirus (bat-SL-CoVZC45, accession MG772933) and SARSCoV (accession NC_004718)) in the E gene" (Broughton et. al, 2020).

In testing this DETECTR system. They used the typical RT-LAMP reaction mixture described in New England Biolabs with 6.5 mM MgSO4 concentration, and a final volume of 10 µl. F3 and B3 primer concentrations were .2 µM. Forward inner and backward inner primers had 1.6 µM concentration, and loop forward and loop backward primers had 0.8 µM concentration. They ran RNA targets synthesized in vitro, suspended in water (without endonucleases) through the DETECTR system, and found that the use of gRNA detecting the N gene was able to detect SARS-CoV-2 with no cross-reactivity, and gRNA detecting the E gene was able to detect SARS-CoV-2 with the cross-reactivity suggested by how the E-gene gRNAs were designed. According to the methods section on page 875, the amplification was run at 62°C for 20 to 30 minutes. The cleavage reaction with Cas12 was then run at 37°C for 10 minutes. The mixture for the cleavage reaction was formed by incubating 50nM LbCas12a with 62.5nM gRNA in 1× NEBuffer 2.1 at 37°C for 10 minutes. After the RNA-protein complex was formed, the lateral flow cleavage reporter was added so that the final concentration is 500 nM.

Afterward, they optimized the DETECTR assay with E gene, N gene, and human RNase P gene (which served as a control). The amplification reaction was ran at 62°C for 20 to 30 minutes, and the Cas12 cleavage reaction at 37°C for 10 minutes. However, the researchers are not clear on how this optimization was determined. In using this test, the researchers noted that detection via both E gene and N gene is considered positive, detection via E gene or N gene is presumptive positive. If only detections via the RNase P gene occur, it is negative. If no detections occur, the test is a "QC failure" (Broughton et. al, 2020). It was unclear if use of this result "matrix" necessitates running 3 assays simultaneously, one for E gene, N gene, and RNase P gene (Fig 5).

N gene	E gene	RNase P	Result
+	+	+/–	SARS-CoV-2 positive
+	–	+/–	Presumptive positive
–	+	+/–	Presumptive positive
–	–	+	SARS-CoV-2 negative
–	–	–	QC failure

Fig 5. Chart describing the interpretation of the DETECTR lateral flow assay.

By running a lateral flow test on the resulting mixture in which uncleaved signaling reporters are collected at the first line and cleaved molecules are collected at the second line, positive and negative results can be determined.

To compare readout signal strength between lateral flow readout and fluorescence methods, DETECTR was carried out using N gene primers and "identical amplicons" (Broughton et. al, 2020). At 0, 2.5, 5, and 10 minutes, the readouts were checked. It was found

that fluorescence was visible in less than a minute and lateral assay produced results "within 5 minutes" (Broughton et. al, 2020). In comparing the fluorescence DETECTR assay to the CDC assay by testing the assays using serial dilutions of nucleoprotein RNA, the Limit of Detection for the CDC assay was 1 copy per µl reaction, and for the fluorescence assay, it was 10 copies per µl reaction.

In testing the ability of the DETECTR assay to detect from the raw matrix (nasopharyngeal swabs from asymptomatic donors) placed in UTM or phosphate-buffered saline, the researchers found that the LoD worsened "at reaction concentrations of ≥10% UTM and ≥10% phosphate-buffered saline by volume" to "15,000 [in UTM] and 500 copies per µl [in phosphate-buffered saline" (Broughton et. al, 2020).

To test agreement between lateral flow DETECTR and fluorescence DETECTR, 11 respiratory swab samples were collected from positive COVID-19 patients. 12 respiratory swab samples were collected from patients with influenza or "common human seasonal coronavirus infections" (Broughton et. al, 2020). Out of the 11 COVID-19 samples, 9 were detected as positive by the tests. The two which were not detected were checked to be below the LoD. The agreement rate was 23/24 or 95.8% and the 24th sample is the no-template control presumably (Fig 6).

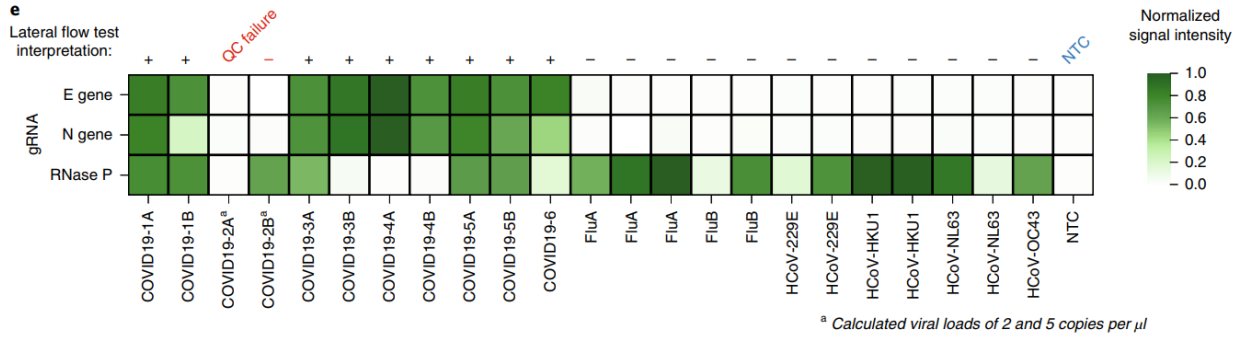

Fig 6. Lateral Flow Test Results and Interpretation for 11 COVID-19 respiratory swab samples, 12 influenza or "common human seasonal coronavirus infection" respiratory swab samples, and one no-template control (Broughton et. al, 2020).

In one last test, the fluorescence DETECTR was used on 60 nasopharyngeal swab samples "from patients with acute respiratory infection" (Broughton et. al, 2020). 30 were tested as positive, 30 were tested as negative. Out of the 83 samples overall, (presumably including the 23 described above) there was a negative predictive agreement rate of 95% and a positive predictive agreement rate of 100% with the CDC assay.

Recently, scientists have engineered specific Cas12a enzymes that increase the efficiency of RT-LAMP testing. For example, scientists have engineered a Cas12a variant that is functional under a very broad set of temperatures, which allows for the entire RT-LAMP workflow to occur in a single heat block. In another study, scientists used Cas12a enzymes for a contamination-free visual detection of COVID-19. In other words, the scientists discovered a method that has high specificity for detecting only COVID-19 RNA molecules. In a laboratory setting, the idea of engineering enzymes can be used to perform experimentation on COVID-19 virus detection. For instance, a group of researchers placed different sequences of ssDNA or ssRNA (approximately 7-31 nucleotides) to the ends of a crRNA-targeting GFP, a fluorescent protein. This was done in

an attempt to improve the efficiency and specificity of detecting COVID-19 in cells because the nucleotide extensions to the crRNA are used for creating greater cleavage than wild-type, or unedited, cr-RNAs. The GFP was used so that other researchers could pinpoint exactly when cleavage was occurring in the CRISPR-Cas process, and the crRNA was isothermally amplified using RT-LAMP. In addition, the researchers employed variations of the Cas12/13 enzymes (by adding sequences), which they tested for the efficiency of detection and cleavage of SARS-Cov-2 DNA/RNA sequences. They labeled their system ENHANCE, and found that the most effective Cas enzyme at trans-cleavage was LbCas12a. The researchers also prepared a very comprehensive table comparing the effectiveness of all the extensions and types of enzymes they used. Overall, ENHANCE allowed for very clear detection of the virus, and the researchers believe that this system can also be used just as accurately for detecting other viruses in the body. (Source: Enhancement of trans-cleavage activity of Cas12a with engineered crRNA enables amplified nucleic acid detection) This source was used in the research paper as a way to introduce a new system of detecting the COVID-19 virus: ENHANCE. The system is described in greater detail when it is observed that CRISPR enzymes and RT-LAMP combined can help accurate detection of not only this virus, but potentially others too. Engineering Cas12/13 enzymes allow researchers to examine and discover the most effective outcome.

In addition to laboratory experimentation, human subjects are also used to test the efficiency of the Cas12/13 and RT-LAMP system when it comes to COVID-19 detection. 10 patients participated in a trial to assess the effectiveness of the RT-LAMP. They were hospital patients who experienced COVID-19 symptoms and tested positive when given the PCR test. From these 10 subjects, viral RNA was extracted. Using fluorescence as a guideline, the variation in the degree of fluorescence showed researchers optimal temperatures of the samples

and no degree of fluorescence was able to be detected in negative samples. This experiment allowed them to make modifications specific to the COVID-19 virus, and when using RT-LAMP, the researchers addressed that "The RT-LAMP step requires a heater that should have accurate thermal stability, i.e., the temperature at each step should be accurate and consistent without variation". They discuss how they went about mounting a heater in order to improve the accuracy of the COVID-19 RT-LAMP method diagnosis. The experiment was particularly important due to its success, which can be used as a model for not only diagnosing COVID-19, but other viruses in patients soon.

DATA ANALYSIS

In order to effectively analyze the CRISPR and RT-LAMP systems in Covid diagnoses, the factors of sensitivity, accuracy, and overall accessibility are measured in several research studies.

The specificity tests conducted by Wang et. al in the study "opvCRISPR: One-pot visual RT-LAMP-CRISPR platform for SARS-cov-2 detection," found that the one-pot visual RT-LAMP-CRISPR only produced signals when RNA from SARS-CoV-2 was present, not when RNA from SARS-CoV or bat-SL-CoVZC45 (bat SARS-like coronavirus) were present. As the authors note, this suggests that the opvCRISPR test is specific and able to distinguish other viruses like SARS-CoV from SARS-CoV-2. This points favorably to the use of one-pot visual RT-LAMP-CRISPR as a diagnostic test for SARS-CoV-2.

With regards to sensitivity, the researchers found that the opvCRISPR test had a detection limit of 5 copies. The test has comparable levels of sensitivity to PCR testing, and is 10 times more sensitive than an RT-LAMP assay without CRISPR. The fact that amplicons yielded within

20 minutes were below the fluorescence threshold, but could be detected with Cas12a cleavage demonstrates the impact that the cleavage reaction has on increasing the sensitivity of RT-LAMP. This reflects the usefulness of implementing CRISPR-Cas12a in the opvCRISPR test.

With regards to the accuracy of the opvCRISPR test relative to PCR in the trial of 26 infected clinical cases and 24 uninfected cases, the opvCRISPR test essentially had a perfect agreement with PCR in marking the infected samples as positive, and uninfected samples as negative. This test's results thus suggest that in terms of accuracy, the opvCRISPR test is on par with PCR, despite being cheaper and quicker.

While the opvCRISPR test was not explicitly tested in comparison to RT-LAMP tests paired with colorimetric readouts, the authors describe the opvCRISPR test as being more specific, sensitive, and distinguishable than RT-LAMP tests paired with colorimetric readouts. The authors also describe this opvCRISPR test as more efficient than other one-pot tests. While the authors present these benefits of the opvCRISPR test, they also acknowledge that this test still requires a step to extract RNA, which means additional work in a point-of-care context.

Overall, results from the "opvCRISPR: One-pot visual RT-LAMP-CRISPR platform for SARS-cov-2 detection" study suggest that the opvCRISPR test has accuracy on par with RT-PCR, sensitivity on par with RT-PCR, and good specificity, all while being quicker and requiring less sophisticated equipment. This points favorably to the use of opvCRISPR as a diagnostic tool for SARS-CoV-2.

Tests of signal strength reflect that signals are observable faster using DETECT with a fluorescence readout (taking less than one minute) than DETECTR with a lateral flow readout, making fluorescence readouts more desirable in this regard. However, comparing DETECTR (fluorescence readout) with the CDC assay (which uses RT-PCR) shows that CDC has a lower

LoD (1 µl) than that of DETECTR (fluorescence); in terms of sensitivity, DETECTR with a fluorescence readout is not as effective as the RT-PCR CDC assay.

The results showing the worsening of LoD (Limit of Detection) for DETECTR using the raw matrix as input above certain concentrations of UTM or phosphate-buffered saline points to the importance of extracting the desired RNA sample from the raw matrix, which the authors of the original paper also acknowledge as subjecting DETECTR to the same limitations as RT-PCR with respect to RNA sample extraction.

The DETECTR assay using fluorescence readout had a very high agreement rate (95.8%) with the DETECTR assay using lateral flow readout; this suggests that one can flexibility use the fluorescence readout and lateral flow readout without significant concerns regarding consistency of results between readouts. For example, the researchers were then able to proceed to test the DETECTR (fluorescence readout) using a larger clinical sample size "Given the high concordance between lateral flow and fluorescence-based readouts".

The high negative predictive agreement (95%) and positive predictive agreement (100%) suggest high concordance between the CDC RT-PCR assay and DETECTR assay. Considering the practical advantages of the DETECTR assay (isothermal process, fast, reduces need for "complex laboratory infrastructure, etc.), the fact that DETECTR is able to achieve such high concordance suggests its usefulness as a diagnostic tool in point-of-care settings. However, the potential consequences of the slightly worse LoD of DETECR (10 µl per reaction vs CDC assay's 1 µl per reaction) must be considered as well in evaluating the usefulness of DETECTR.

In another research study titled "CRISPR–Cas12-based detection of SARS-CoV-2," it depicts the enhancement of trans-cleavage activity of Cas12a with engineered crRNA enables amplified nucleic acid detection. Scientists have engineered specific Cas12a enzymes that

increase the efficiency of RT-LAMP testing. For example, scientists have engineered a Cas12a variant that is functional under a very broad set of temperatures, which allows for the entire RT-LAMP workflow to occur in a single heat block. In another study, scientists used Cas12a enzymes for a contamination-free visual detection of COVID-19.The scientists discovered a method that has high specificity for detecting only COVID-19 RNA molecules. In a laboratory setting, the idea of engineering enzymes can be used to perform experimentation on COVID-19 detection. For instance, a group of researchers placed different sequences of ssDNA or ssRNA (approximately 7-31 nucleotides) to the ends of a crRNA-targeting GFP, a fluorescent protein. This was done in an attempt to improve the efficiency and specificity of detecting COVID-19 in human cells, since these nucleotide extensions to the crRNA are used for creating greater cleavage than wild-type, or unedited, cr-RNAs. The GFP was used so that other researchers could pinpoint exactly when cleavage was occurring in the CRISPR-Cas process, and the crRNA was isothermally amplified using RT-LAMP. In addition, the researchers employed variations of the Cas-12/13 enzymes (by adding sequences), which they tested for the efficiency of detection and cleavage of SARS-Cov-2 DNA/RNA sequences. They labeled their system ENHANCE and found that the most effective Cas enzyme at trans-cleavage was LbCas12a.

When determining the ideal Cas enzyme to use, researchers relied on the p-test, highlighting which engineered enzymes were significant versus not significant, with the end result of LbCas12a having a p-value<0.05. In addition, modifications were added to the LbCas12a in an effort to improve the accuracy of trans-cleavage, with the crGFP + 3’DNA7 being the best option, as the RFP value and Fold (normalized) measurement increased over time. Determining the optimum enzyme for this detection system without increasing cost and materials can prove to be highly beneficial in future research.

Furthermore, improved specificity and sensitivity of trans-cleavage with ENHANCE resulted from experimentation. There was approximately 5.4-fold and 3.4-fold and higher trans-cleavage activity than the “unedited” or “wild-type” cRNAs used. With regard to sensitivity, this was determined within 30 minutes, whereas in the previous research study the system designed was effective within 20 minutes. However, the crGFP + 3’DNA7 showed detection was within 3-300 copies of RNA.

Overall, ENHANCE allowed for very clear detection of the virus, and the researchers believe that this system can also be used just as accurate for detecting the presence of not only COVID-19, but other diseases as well.

DISCUSSION

Though many of the experiments observed claim to yield results with high specificity, some fail to address possible errors in their experimentation. A big issue with testing the efficacy of COVID-19 testing methods is the possibility of contamination while running tests. Specifically, trials are prone to aerosol contamination during the transfer process between the LAMP amplification and further processes. False positives caused by aerosol contamination occur most frequently after having to open the test tube lid to add CRISPR reagents (Chen et. al, 2020). Thus, if this issue is not addressed throughout the experimental process, then the final testing result can yield false positives. In addition, some experiments that claim to only utilize LAMP amplification to yield high specificity are prone to false positives as well. According to the study “Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing”, researchers found that using the “LAMP [procedure] alone can produce nonspecific signals” (Juong et. al, 2020). This could later result in false results” (Ooi et. al 2021).

Furthermore, many experiments fail to verify the specificity of their experimentation by testing it with other common viruses. Multiple engineering experiments tend to focus on only the efficacy of the product on COVID-19 only. They fail to bring in other common viruses such as the flu, cold, and other forms of COVID-19 to test alongside the current coronavirus. This poses a threat to the validity of the specificity of the researchers' products because though the product may be able to detect the presence of COVID-19, this does not consider whether it is able to identify only COVID-19 viruses.

Adding on, multiple experiments seemingly only test the most current COVID-19 variant of its experimentation. Because COVID-19 is able to spur up different variants so quickly, it is important that the testing methods are able to adapt and identify not only the current coronavirus variant but derivatives of it too. Though this would be hard to test without having actual derivatives of the virus, it is an important factor to consider when analyzing the validity of each CRISPR-Cas12 or CRISPR-Cas13 level of specificity.

Finally, it is important to consider the ethical issues with COVID-19 experimentation. A major concern with viral testing is the high risk of exposure for both researchers and treatment groups. Especially when working under experimental conditions, the risk of researchers catching COVID-19 or spreading it to treated patients is heightened. It is important that the researchers take precautionary measures throughout the entire process in order to mitigate the spread of the virus. Viral experimentation itself is a risk to the public, which is why COVID-19 experimentation is a big concern to the public health. The final concern with the researchers'

data is that it is subject to a biased interpretation of results or exaggerated write-ups due to pressure from governments and the public for scientists to push out new information about the virus. After analyzing multiple published papers, many experiments were funded by federal governments, organizations, or groups. Though each article claims that there is no competing financial interest while conducting their experiments, pressure from companies and the public could bias the interpretation and write-up of results. Especially during the height of the pandemic, people demanded more information on the virus as soon as possible; this could have led to less thorough analyses or exaggerated reports to please the public.

CONCLUSION

As it has previously been suggested in earlier academic publications, the use of RT-LAMP procedures and the CRISPR-Cas system in conjunction with each other can potentially offer positive results in terms of yielding fast and accurate COVID-19 results. Given the infectiousness of COVID-19 and the technicalities of conducting PCR tests, it is imperative that within the current climate that another type of COVID-19 test is developed that increases the turnover time of test results but also minimizes potential inaccuracies.

Despite the optimism surrounding many RT-LAMP and CRISPR-Cas system clinical trials that were conducted under high levels of specificity, some experiments indicate that there is still a lot of research necessary to come to a complete conclusion regarding the COVID-19

testing capabilities of the RT-LAMP and CRISPR-Cas system method. This concern is reasonable in that it addresses the possibility of aerosol contamination during RT-LAMP/CRISPR-Cas amplification, the RT-LAMP procedure potentially providing nonspecific signals resulting in false positive results, and with multiple clinical trials and investigations needed to be conducted due to emerging COVID-19 variants. Furthermore, with the pandemic continuing to affect countries' academic centers, it is plausible for governments and scientists to be incentivized to work towards more public health advancements moving forward in terms of more effective testing avenues and vaccine treatments.

Needless to say, given the potential groundbreaking change that the RT-LAMP and the CRISPR-Cas system could have, scientists and academic research institutions are still exploring both of these technologies in conjunction with each other in the hope that they may change the current landscape of COVID-19 testing. Going forward into the future, it is thus still an important focus for governments & scientists to develop a COVID-19 test that can be processed quickly without the need of many experienced medical personnel and is as or more accurate that PCR testing. If this is not achieved in the near future, then it will only become tougher to fight against the COVID-19 pandemic and its ever-changing variants Thus, it can be concluded that as a result of the reported benefits and issues that exist with the RT-LAMP and CRISPR-Cas system method of COVID-19 testing, more research is still needed regarding its practical applications.

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